

Po1_1.03

Effect of interfragmentary stimuli on repair tissue formation in a bone healing model with monotonically increasing interfragmentary strain

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Abstract

The formation of fracture callus during secondary bone healing is influenced by local mechanical conditions in the fracture gap. This study investigates how low to medium magnitudes of interfragmentary strain, and the temporal distribution of stimulation impact the formation of fracture repair tissue in a bone healing model with monotonically increasing interfragmentary strain.

An experimental tibial defect was created in ten sheep and was stabilized with an active external fixator that induced controlled interfragmentary motion with a strain gradient ranging from 2.5% to 25%. The sheep were randomly divided into two groups: one received immediate stimulation (from day 1 post-op), and the other received delayed stimulation (from day 22 post-op). The animals were euthanized five weeks post-op, and the explanted tibiae were scanned using high-resolution computed tomography (HRCT). HRCT scans were sliced at areas corresponding to different strain levels. At each strain level, we evaluated the area and density of calcified tissue in the intracortical region, as well as the span of the periosteal callus.

The largest area and density of calcified tissue in the intracortical regions were observed under the lowest strain conditions and significantly decreased as strain increased ($p \leq 0.015$). In contrast, the span of the periosteal callus increased with strain ($p < 0.001$), being significantly larger for the group that received immediate loading ($p < 0.01$).

Our study illustrates the mechanism of bone healing in relation to interfragmentary strain and the temporal distribution of stimulation. When the strain in the fracture gap is sufficiently low, calcified repair tissue can form directly within the gap. In contrast, when strain is too high, external callus develops proportionally to the interfragmentary strain in an attempt to reduce it, thereby enabling calcification and promoting bone union. The superior callus formation in the immediate stimulation group indicates the importance of mechanical stimulation in the early healing phase for prompt formation of fracture repair tissue and bone consolidation.

Po1_1.05

Optimizing RNA *In Situ* Hybridization for Gene Expression Mapping in Mouse Fracture Healing

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Abstract

Background: Fracture non-unions pose challenges in the field of orthopaedics due to their complex genetic, metabolic, and mechanical origins. Understanding the molecular mechanisms involved in fracture healing and non-union is essential for developing effective therapies. The dense bone matrix has historically limited the use of spatially resolved "omics" technologies, such as RNA in situ hybridization (RNA ISH), which are critical for mapping gene expression within bone. This study compares two preprocessing and decalcification methods for Formalin-Fixed Paraffin-Embedded (FFPE) mouse bone: De Charleroy *et al.* (2021) and Wehrle *et al.* (2024). Using RNAscope 2.5 HD duplex detection kits, the study aims to identify a protocol compatible with both RNA ISH and spatial transcriptomics (ST).

Methods: The RNA ISH protocol is established using the RNAscope 2.5 HD duplex detection kit (ACD Bio) to visualize RNA expression in FFPE samples on mouse femurs from previous fracture healing studies (GR/22/2022, GR/27/2023). Double-z stacked probe hybridization enhances sensitivity and specificity to overcome challenges posed by the dense bone matrix. RNA quality (DV200, TapeStation, Agilent), and tissue integrity are assessed post-decalcification. Two decalcification methods (n=2/group) were compared: 12.5%Ethylenediaminetetraacetic acid (EDTA), which offers gradual decalcification (10 days), and Morse's Solution, which achieves faster results (3 days) but risks RNA degradation.

Results: Mouse femur samples decalcified with EDTA 12.5% consistently yielded DV200 values (47.84%, 48.36%) > 30% threshold, sufficient for ST. These findings align with Wehrle *et al.* (2024), supporting EDTA 12.5% as a reliable method for preserving RNA quality while maintaining tissue structure. Conversely, Morse's solution resulted in DV200 values (16.54%, 12.00%) < 30%, indicating RNA degradation and potential low applicability of samples to FFPE-based ST approaches.

Conclusion: Preliminary findings suggest decalcification with 12.5% EDTA, based on Wehrle *et al.* (2024), results in RNA quality exceeding the 30% threshold recommended for ST. However, Morse's solution requires further optimization to achieve a better balance between rapid decalcification and RNA preservation. The study will be supplemented with additional samples, targeted towards establishing a pre-treatment protocol enabling both ST and RNA ISH on the same specimen. Future research will also explore decalcification

conditions to enhance RNA ISH applicability and extend this protocol to clinically relevant conditions such as Fracture-Related Infections (FRIs).

Acknowledgements: This project is funded by AO Trauma. PhD salary (N.G.) covered by the SNSF 213520.

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Po1_1.08

Characterization of the Osteocyte Lacunocanalicular Network in Subchondral Bone of Healthy Mice

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Abstract

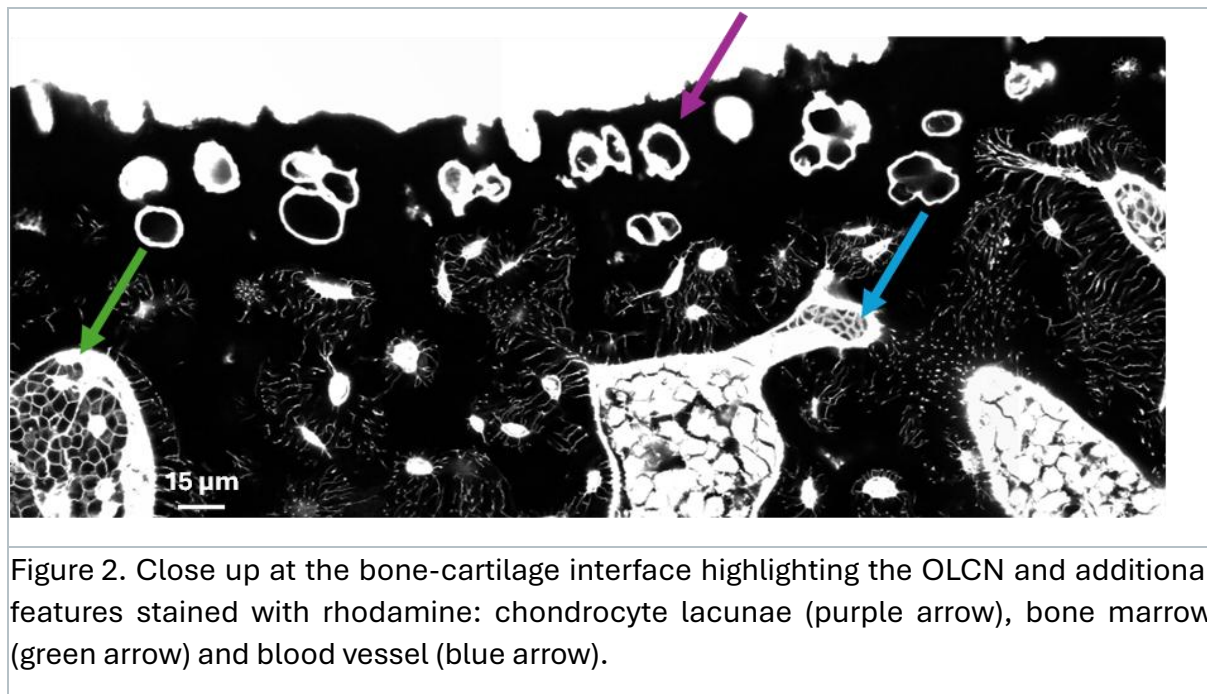
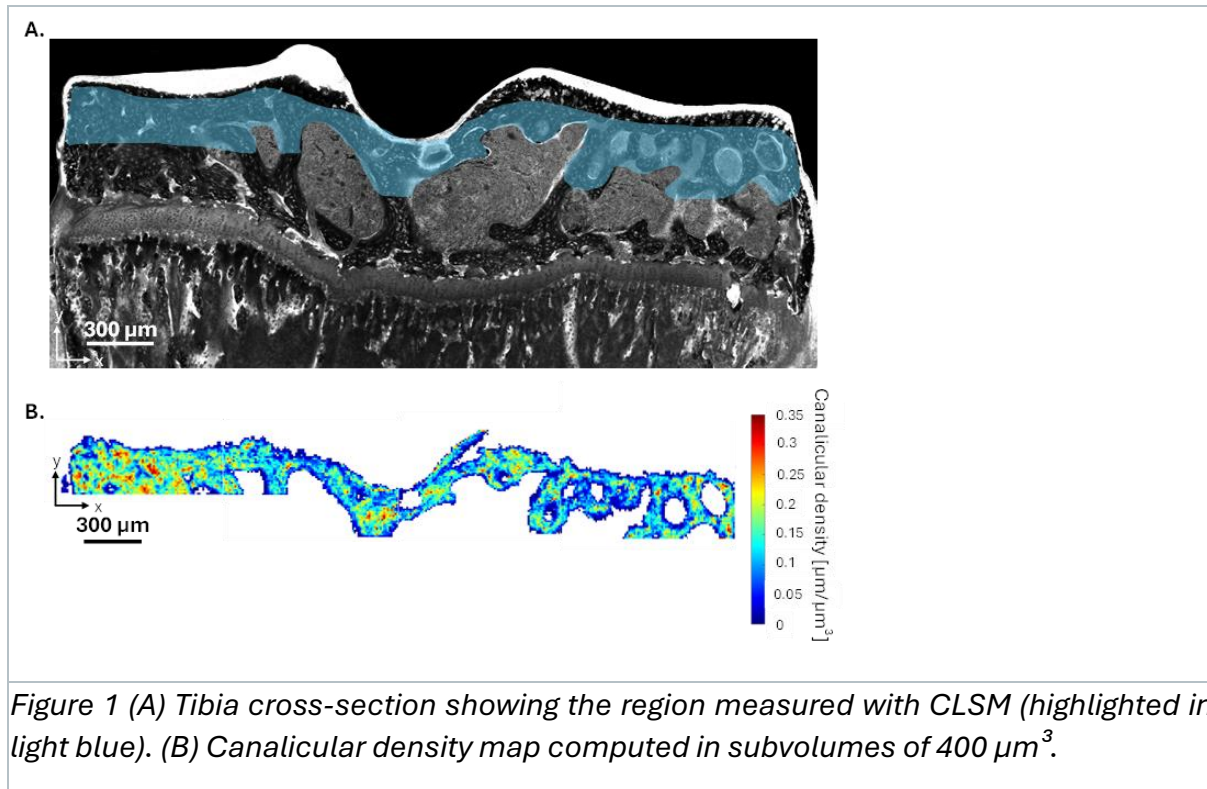
When bone and cartilage are joined together, both tissues present specific adaptation strategies to solve the biomechanical and biological dissimilarities between them. The bone that articulates in the joint is called subchondral bone (SB) and is separated from the articular cartilage by a region of mineralized cartilage. At the cellular level, mineralized cartilage is populated by chondrocytes whereas SB contains osteocytes. The latter are highly interconnected through the osteocyte lacunocanalicular network (LCN). Osteocytes are believed to regulate the biological processes of bone remodeling and mineralization [1]. Changes in SB are involved in joint aging and pathologies, such as osteoarthritis (OA). Common modifications observed in OA are subchondral thickening and increased subchondral remodeling [2]. The specific architecture of the LCN in the SB remains poorly characterized, limiting our understanding of its role in subchondral modifications. This study focuses on the LCN of SB and on the possible connections between osteocytes in SB and chondrocytes in mineralized cartilage. We analyzed a 10-week-old male C57Bl/6J mouse tibia and LCN was stained with rhodamine and imaged using confocal laser scanning microscopy (Stellaris 8, Leica) at a lateral resolution of 0.18 μm over a depth of 40 μm . Network analysis was performed with the dedicated open-source software TINA [3]. Canalicular density (Ca.Dn) exhibited high spatial heterogeneity, with lower Ca.Dn observed near the bone-cartilage and the bone-marrow interface (Fig. 1). Numerous stained chondrocyte lacunae (Fig. 2) were found over the full width of the articular cartilage, irrespective of their proximity to SB. Our initial results provide reference data for future work comparing healthy and post-traumatic OA scenarios.

Acknowledgement

This project has received funding from the FWO and F.R.S.-FNRS under the Excellence of Science (EOS) programme (EOS No. 40007553)

References

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Po1_1.09

Cortistatin prevents glucocorticoid-associated osteonecrosis of the femoral head via the GHSR1a/Akt pathway

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Abstract

Objective: Long-term use of glucocorticoids (GCs) is known to be a predominant cause of osteonecrosis of the femoral head (ONFH). Moreover, GCs can mediate apoptosis of various cell types by exaggerating oxidative stress. The objectives of this research were to elucidate the potential function and underlying mechanisms of CST in the ONFH process.

Material and Methods: In this study, we detected that the CST expression levels were diminished in patients with ONFH compared with femoral neck fracture (FNF). In addition, a GC-induced rat ONFH model was established, which impaired bone quality in the femoral head. Then, administration of CST attenuated these ONFH phenotypes. Furthermore, osteoblast and endothelial cells were cultured and stimulated with dexamethasone (Dex) in the presence or absence of recombinant CST.

Results: Dex induced impaired anabolic metabolism of osteoblasts and suppressed tubeformation endothelial cells, while additional treatment with CST reversed this damage to the cells. Moreover, blocking GHSR1a, a well-accepted receptor of CST, or blocking the AKT signaling pathway largely abolished the protective function of CST in Dex-induced disorder of the cells.

Conclusion: We indicate that CST has the capability to prevent GC-induced apoptosis and metabolic disorder of osteoblasts in the pathogenesis of ONFH via the GHSR1a/AKT signaling pathway.

Po1_1.11

Does Freeze-Thawing Change the Metabolomic Active of Platelet-Rich Plasma?

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Abstract

Platelet-rich plasma (PRP) is often frozen and thawed for repetitive clinical use. This study examines whether freezing and thawing alter PRP's metabolomic activity. Active PRP, prepared using activators like calcium chloride or thrombin, releases growth factors such as PDGF, TGF- β , and VEGF. In contrast, conventional PRP relies on natural activation at the application site. The metabolomic differences between active and conventional PRP types were investigated.

Blood from six male volunteers was used to prepare two conventional PRP types (leukocyte-rich [L-PRP] and leukocyte-poor [P-PRP]) and three active PRP types (PRGF, active PRP G, and active PRP S). Samples were prepared fresh or stored at -20°C for one week. After protein precipitation and metabolite extraction, samples were analyzed using Q-TOF LC/MS. Data were processed with MZmine and MetaboAnalyst 6.0, and metabolite identities were confirmed using HMDB and KEGG databases.

Metabolomic analysis revealed significant changes in all PRP types after freezing and thawing, except for active PRP-S (Figure 1). L-PRP showed the most differentiation, with over 250 peaks altered after freezing. Key metabolites like glucose, phosphocreatine, and citric acid, crucial for energy metabolism, decreased across PRP types. Antioxidants such as glutathione and biliverdin also declined, potentially compromising PRP's regenerative capacity and increasing oxidative stress.

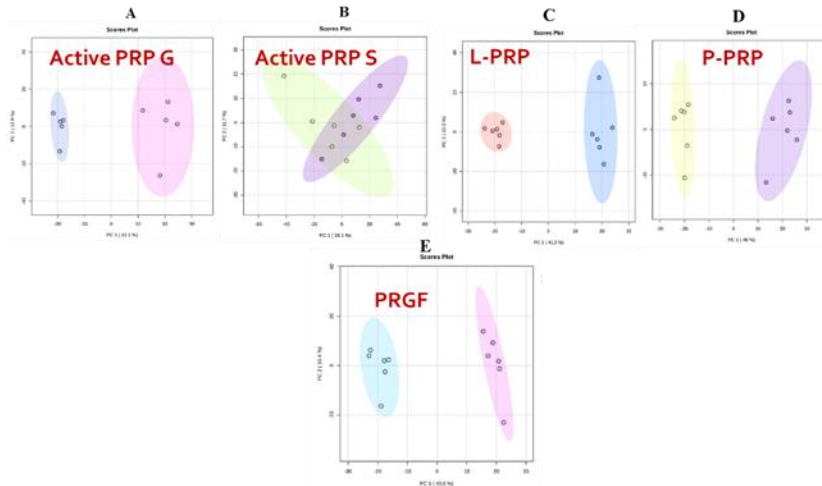


Figure 1. PCA plots for fresh and frozen PRP samples.

The freeze-thawing process led to a general decrease in metabolites, indicating a lack of stability. Significant reductions were observed in energy metabolism components (glucose, phosphocreatine, citric acid) and antioxidant defense systems (glutathione, biliverdin). This suggests that frozen PRP may have a lower regenerative capacity compared to fresh PRP. Furthermore, when considering the aspects of degeneration and inflammation, the decrease in metabolites and antioxidants associated with oxidative stress may lead to an increase in inflammatory processes or make their control more challenging. The reduction in neurotransmitter derivatives such as dopamine and catecholamine metabolites like 3,4-dihydroxyphenylglycol may also impact the regulation of immune and inflammatory responses.

In conclusion, the metabolomic differences among PRP types significantly impact clinical efficacy and storage stability. Choosing the right PRP type for the patient's needs and optimizing storage conditions can enhance treatment outcomes. This study provides valuable insights into PRP storage stability, aiding physicians in developing more effective treatment strategies.

Acknowledgements

This research was supported within the scope of TÜBİTAK 1001-Scientific and Technological Research Projects Support Program (#222S093).

Ethics Statement

This study was approved by Hacettepe University Non-Invasive Clinical Research Ethics Committee (20.09.2022/Approval no:2022/14-57)

Po1_1.12

Role of the β_2 -Adrenoceptor in Chondrocytes for Long Bone Growth and Fracture Healing in Mice

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Abstract

Adrenergic signaling is known to influence bone metabolism and fracture healing. We recently could show that catecholamines impair endochondral ossification during bone growth and fracture healing. The β_2 -adrenoceptor plays an important role in mediating the effects of catecholamines on bone. Mice with a systemic or an osteoblast-specific knockout of the β_2 -adrenoceptor show an increased bone mass. The aim of this study was to investigate the effects of a chondrocyte-specific knockout of the β_2 -adrenoceptor on long bone growth and fracture healing in mice.

Male and female $Adrb2^{fl/fl}/Col2a1-Cre^+$ ($Adrb2^{Col2a1-Cre}$) mice with a conditional knockout of the β_2 -adrenoceptor in chondrocytes and their Cre^- littermates ($Adrb2^{fl/fl}$) were euthanized at the age of 11-12 weeks. Femur lengths were measured and the growth plates were analyzed using histological stainings. To investigate the plasma catecholamine concentration, a 3-CAT ELISA was performed. For analyzing fracture healing, another set of male $Adrb2^{Col2a1-Cre}$ received a femur osteotomy stabilized with an external fixator. Fracture healing was analyzed on day 10 and 21 after osteotomy using histology, μ CT and 3-point-bending testing. Data analysis: Students t-test. $p < 0.05$. $n = 4-6$.

Male and female $Adrb2^{Col2a1-Cre}$ mice showed shorter femora (males: 14.9 vs 15.8 mm, $p < 0.0001$; females: 14.2 vs 14.8 mm, $p = 0.021$) compared to the $Adrb2^{fl/fl}$ mice. The growth plate thickness was unaltered, but the males showed an enlarged hypertrophic zone (31.9 vs 25.2 μ m, $p = 0.009$) with an increased amount of Col10⁺ hypertrophic chondrocytes (35.1 vs 23.2%, $p = 0.031$). This was not observed in the females, although there was no difference in the plasma catecholamine concentration. On day 10 after fracture, cartilage area in the fracture callus was by trend reduced (19.8 vs 38.0%, $p = 0.083$). 21 days post-fracture, cartilage area was increased (24.0 vs 2.6%, $p = 0.03$) and relative bone volume was reduced (6.5 vs 13.7%, $p = 0.004$) accompanied by a decreased relative flexural rigidity of the fractured femora (26.8 vs 52.58%, $p = 0.015$).

Our data indicate an important role of the β_2 -adrenoceptor in chondrocytes for long bone growth and fracture healing. The reduced bone growth might be due to an accelerated

transition of proliferative to hypertrophic chondrocytes in the growth plate. First RNASeq data from an additional in vitro experiment support this hypothesis. The less pronounced effects in females could possibly be caused by an influence of sex hormones. The disturbed fracture healing might be due to an impaired cartilage formation based on the same mechanisms as in the growth plate. Further research is necessary to unravel the underlying mechanisms.

Po1_1.15

Regulating stem cell growth factor- β fine-tuned balance of osteogenesis and adipogenesis of mesenchymal stem cells endows Statins with new anti-osteonecrosis effect

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Abstract

Dyslipidemia has been implicated in osteonecrosis through some clinical studies. However, a direct causal relationship between hyperlipidemia and osteonecrosis remains unconfirmed, and whether lipid-lowering agents could be used to treat osteonecrosis remains unclear. This study aimed to investigate the causal role of lipid traits in osteonecrosis using Mendelian randomization (MR) analysis, assess the potential effects and mechanisms of lipid-lowering drug targets on osteonecrosis risk, and validate these findings through experimental approaches. Genetic data from genome-wide association study (GWAS) were used to analyze lipid traits and drug target gene variants. The FinnGen osteonecrosis dataset and expression quantitative trait loci (eQTL) data were analyzed using colocalization and mediation MR to validate findings and explore mediators. Statin effects were further studied in a rat model of steroid-induced osteonecrosis and *in vitro* cell models. MR analysis revealed a significant association between LDL-C and increased osteonecrosis risk. Genetic mimicry of HMGCR inhibitors was associated with reduced osteonecrosis risk, which was validated through colocalization. Stem cell growth factor- β (SCGF- β) was identified as a mediator of 2% of HMGCR inhibitors' effect on osteonecrosis risk. Further studies confirmed simvastatin's alleviating effect on SONFH, suggesting that simvastatin promotes osteogenesis and inhibits adipogenesis of mesenchymal stem cells (MSCs), partly mediated by SCGF- β upregulation, which activates the Wnt signaling pathway. Our findings support dyslipidemia as a causal factor for osteonecrosis, highlighting HMGCR as a promising therapeutic target. The anti-osteonecrosis effect of statins may be mediated through the regulation of MSCs' osteogenesis and adipogenesis via SCGF- β .

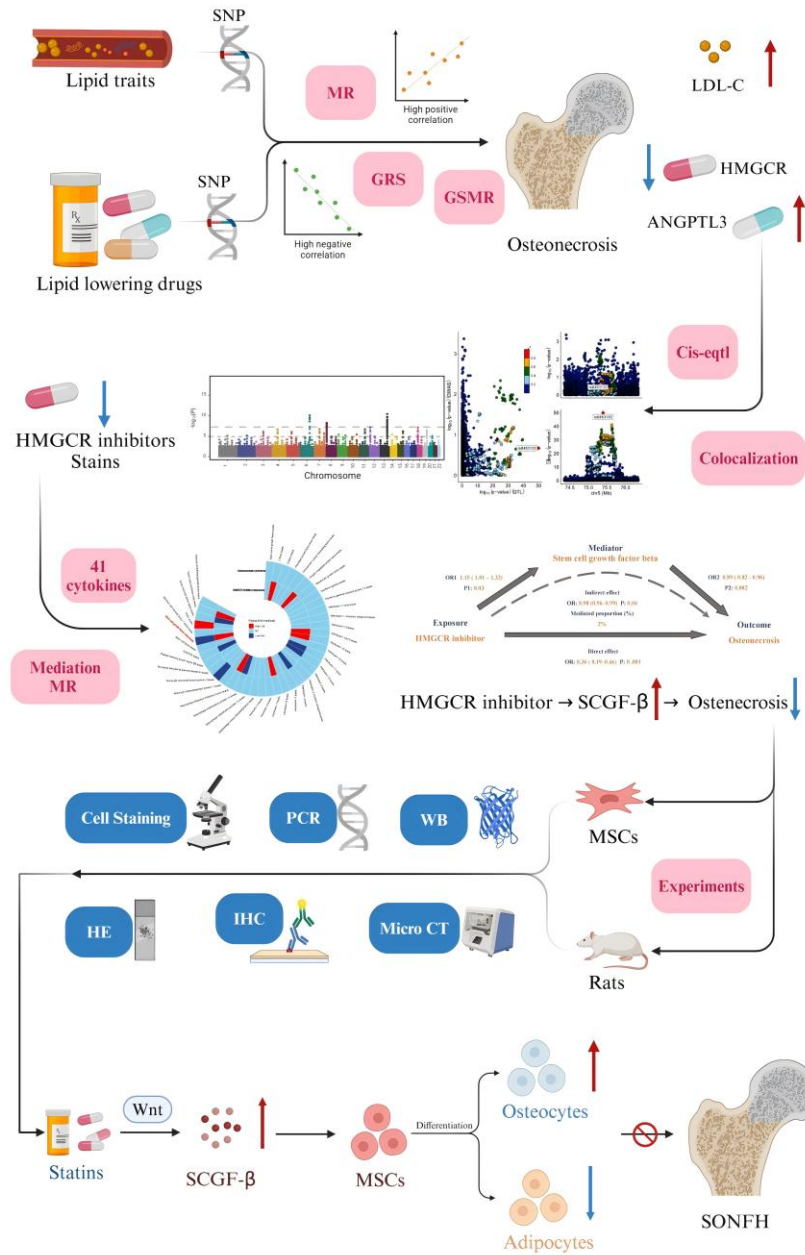


Figure 1. The flowchart of study design

Po1_1.17

Differential Pain Resolution Behaviors Following Fracture in Rodents Based on Sex and Fracture Stability

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Abstract

Introduction: Bone fractures are common orthopedic injuries, yet research on post-fracture pain in preclinical models remains limited. Existing rodent studies primarily use the von Frey technique or gait analysis, but these methods lack sensitivity to detect the full spectrum of pain following fracture. To address this gap, we developed novel machine learning algorithms to objectively monitor behavioral changes after fracture using the Blackbox system. This study investigates how different surgical techniques and sex impact post-fracture kinematics. We hypothesize that the Blackbox system, combined with our high-throughput analysis, will identify gait-related changes sensitive to fracture injury and recovery.

Methods: We quantified behavior and kinematics in adult C57BL/6J mice with tibia fractures that either received intramedullary stabilization (n=5) or no stabilization (n=5), with non-fractured mice serving as controls (n=5). Mice were analyzed at baseline and throughout healing (4 to 35 days post-fracture, DPF) using the Blackbox system, which records animal pose and paw weight-bearing during free movement. Recordings were analyzed using our Paw Analysis Workflow (PAW), a machine learning-based software that tracks changes in paw pressure and kinematic phenotypes. We also examined sex-specific differences in pain response following pin-stabilized fractures using the same workflow, further investigating how biological sex may influence post-fracture recovery dynamics.

Results: Following fracture, both stabilized and unstabilized fracture mice reduced weight-bearing on the injured leg, producing a hindpaw ratio (weight-bearing on fractured hindlimb/weight-bearing on non-fractured hindlimb) less than 1 up to 7 DPF. By 10 DPF, stabilized fracture mice returned to full weight-bearing, whereas unstabilized fracture mice maintained a significantly different hindpaw ratio ($p = 0.0269$). Sex differences were observed in non-fractured mice, with female mice exhibiting higher maximum paw speed ($p < 0.0001$) and stride length ($p < 0.0001$) than males. In fractured mice, females returned to weight-bearing faster than males, while males regained baseline max paw/walking speed more quickly.

Discussion: Our data show that combining the Blackbox system with high-throughput machine learning tools (PAW) significantly improves detection of kinematic changes after

fracture. This approach also reveals subtle sex-specific differences in post-fracture pain behaviors. The Blackbox system enhances the sensitivity and comprehensiveness of pain-related behavior quantification, providing a more effective tool for understanding and addressing post-fracture pain. The ability to capture such detailed behavioral data offers unprecedented insight into the mechanisms underlying fracture recovery and post-injury pain.

Po1_1.18

Gambogic Amide induces Heterotopic Ossification expansion after Soft Tissue Trauma modulating TrkA+ neurons

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Abstract

Heterotopic ossification (HO) is defined as aberrant bone formation in extraskeletal locations. In this process, local stromal cells of mesenchymal origin abnormally differentiate, resulting in pathologic cartilage and bone matrix deposition in soft-tissues including tendons, ligaments, and muscles.

The abnormal generated bone and location induce pain and loss of mobility. In our recent studies, we observed that Nerve growth factor (NGF)-responsive Tropomyosin receptor kinase A (TrkA)-expressing nerves access to soft-tissue trauma sites, showing that this is a necessary feature HO generation.

Here, we evaluated the effects of Gambogic amide (GA), a partial TrkA agonist in peritendinous heterotopic bone after extremity trauma. HO induction was performing using the burn/tenotomy mouse model with or without systemic treatment with GA. HO were followed by an examination of the injury site with radiological imaging (x-Ray and Micro-CT), histology, and immunohistochemistry. Single-cell RNA Sequencing confirmed an increase in neurotrophin signaling activity after HO-inducing extremity trauma. Next, TrkA agonism led to injury site hyper-innervation, more brisk expression of cartilage antigens within the injured tendon, and a shift from FGF to TGF β signaling activity among injury site cells. Nine weeks after injury, this culminated in higher overall levels of heterotopic bone among GA-treated animals. In summary, these studies further link injury site hyper-innervation with increased vascular ingrowth and ultimately heterotopic bone after trauma. In the future, modulation of TrkA signaling may represent a potent means to prevent the trauma-induced heterotopic bone formation and improve tissue regeneration.

Po1_1.19

The Biofiligree® concept: innovative approach to bone fracture healing

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Abstract

Surgical procedures for bone fracture healing are well-established and highly effective in clinical practice today. However, there remains space for innovation, particularly in enhancing the patient experience. By merging medicine and engineering with art and design, new possibilities emerge for fostering psychotherapeutic benefits alongside physical healing. One such innovation is the Biofiligree® concept, which transforms osteosynthesis plates into biofiligree plates for both healing and artistic self-expression. With biofiligree plates (figure 1), inspired by traditional Portuguese filigree, patients can actively participate in their recovery since they have the possibility to select unique geometries, patterns, or engravings. Once the bone fracture has healed and the plates are removed, these intricately designed plates can be transformed into wearable jewellery, serving as a sentimental item of the healing process.

To assess the biomechanical viability of these plates, a proof of concept was conducted using finite element analysis on a fractured calcaneus. The strains were compared and validated with those obtained with an experimental model (figure 2) for filigree and conventional plates. Results showed that both plates can sustain the load without material failure. The filigree plate exhibited slightly lower fixation capacity - with bone fragment displacement at the fracture line being 5% higher than that of the commercial plate. Thus, osteosynthesis plates with filigree patterns represent a viable alternative for calcaneus fractures. Beyond their functional performance, these plates introduce a unique therapeutic dimension. By integrating art into medical treatment, patients can benefit from a more personalized and emotionally meaningful recovery process. This approach opens new pathways for enhancing patient engagement and well-being during the recovery process.

Acknowledgments: This study was funded by Fundação para a Ciência e a Tecnologia by projects EXPL/ART-DAQ/1347/2021 and 2023.15616.PEX.

Po1_1.21

The Role of Mineral Heterogeneity, Trabecular Orientation, and Collagen Fibers Arrangement on Tissue-Level Trabecular Bone Mechanics

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Abstract

Trabecular bone is a porous tissue, with a multiscale heterogeneous and anisotropic microstructure. At the architectural length scale, it features a complex network of beams (i.e. trabeculae). At the tissue level each trabecula comprises multiple bone packets, with different shapes, mineral content, and collagen arrangement [1]. The influence of material heterogeneity and structural anisotropy on the mechanical behaviour of trabecular bone remains poorly understood. Here, tissue-level mechanical properties of trabecular bone were considered in individual bone packets, in relation to local mineral content and collagen organization. A post-mortem trabecular bone sample was extracted from the iliac crest of a healthy 93 y.o. woman. Quantitative backscattered electron imaging (qBEI) generated 2-dimensional maps of the mineral content, while micro-computed tomography (micro-CT) provided 3D information on the trabecular network below the exposed surface. By superimposing the two techniques, trabeculae showing similar Ca content and oriented parallel and perpendicular to the qBEI plane were selected, to address microstructural anisotropy. Indentation elastic modulus was measured on the selected trabeculae using nanoindentation (nIND). Second harmonic generation microscopy (SHG) was employed to assess collagen organization qualitatively. Results from qBEI and nIND for all indents are shown in Fig. 1, demonstrating that for comparable values of mineral content, the measured elastic modulus tends to be higher for perpendicular trabeculae, which were in average 1.38 stiffer than horizontal trabeculae. SHG images (Fig. 2) reveal well-defined lamellar patterns in both trabeculae. Discontinuities at cement lines separating different bone packets were observed, with some neighbouring packets displaying differently oriented lamellae. Notably, SHG images of perpendicular trabeculae showed a weaker signal (darker grey levels) compared to parallel trabeculae, indicating a higher degree of out-of-plane fibril orientation, as SHG signals arise mainly from in-plane collagen fibril [2]. Thus, collagen fibrils are predominantly aligned along the trabecular axis, this is consistent with the mechanical anisotropy shown by nIND, as reported in literature [3]. These results will inform a computational model to unravel the interplay between trabecular microstructure and material heterogeneity in trabecular bone mechanics.

[1] Smith et al., *Journal of Biomechanics* 43, 2010

[2] Collins et al., *Materialia* 9, 2020

[3] Reznikov et al., *Bone* 71, 2015

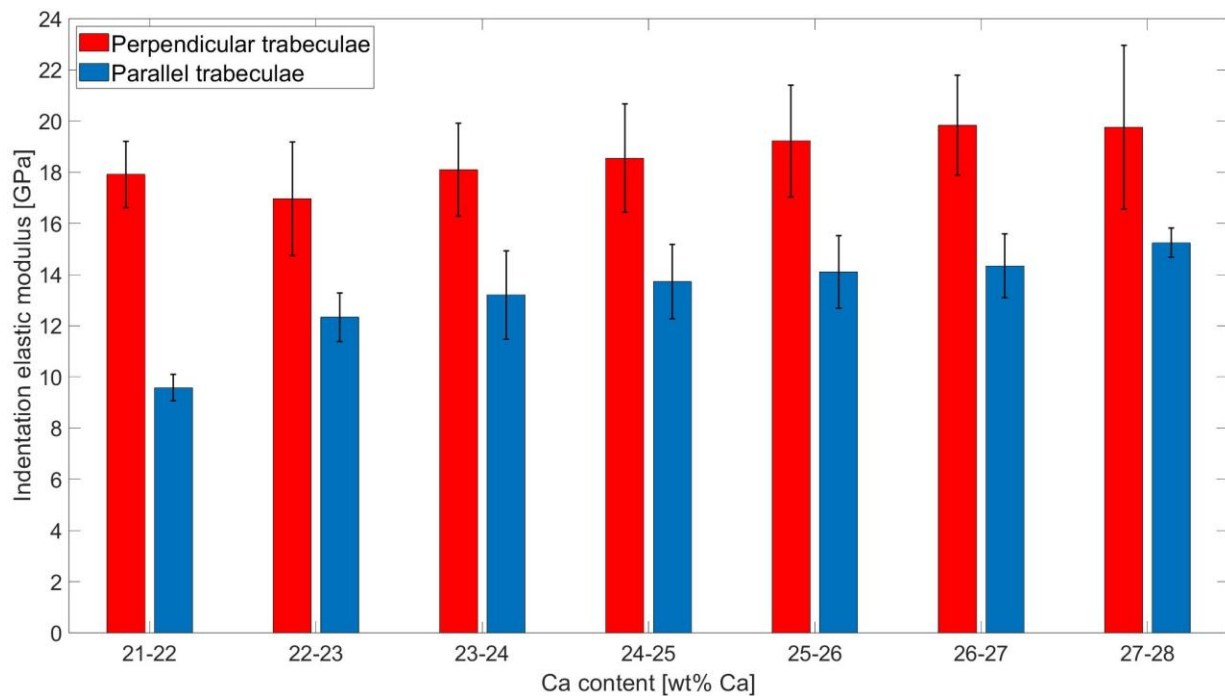


Fig. 1: Average indentation modulus for specific Ca content values. Indents are grouped in two categories: perpendicular and parallel trabeculae. Data shown as mean value $\pm$ standard deviation.

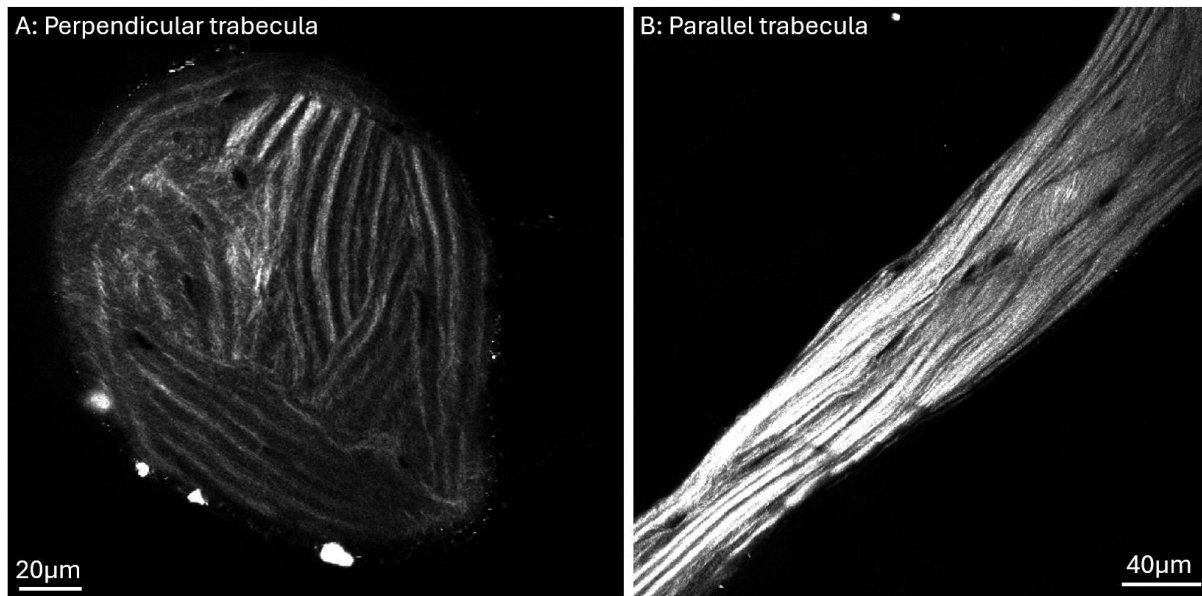


Fig. 2: SHG images of two analyzed trabeculae oriented roughly (A) perpendicular and (B) parallel to the imaging plane.

Po1_1.20

Understanding the behaviour of macrophages and vascular cells on shifted laser surface textured titanium discs for implant integration

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Abstract

Objective: Hip and knee joint arthroplasties require revision due to early or late implant loosening. Micro- and nano-texturing has been shown to enhance bone regeneration and thereby implant integration with the surrounding tissue. An essential part of this process are the interactions between osteoprogenitors, vascular and immune cells. How vascular and immune cells are influenced by these textured surfaces remains to be studied in detail. The present work investigated the behaviour of vascular cells and macrophages on titanium (Ti) surfaces that are textured using shifted Laser Surface Texturing (sLST) followed by laser ablation with calcium silicide (CaSi₂) compared to smooth Ti surfaces.

Methods: Ti grade 2 disks (d/h: 12 mm/1.2 mm) were subjected to shifted Laser Surface Texturing (SPI G3, beam: 1064 nm, pulse duration: 200 ns) to generate open pores (l/w: 500µm, h: 300µm) and micro-nano-texturing (LT-Ti). A set of LT-Ti and smooth-Ti (SM-Ti) discs were coated with calcium silicide (CaSi₂) by laser ablation either in vacuum or ethanol (100%) colloidal dispersion (emulsion) form. Uncoated discs were used as controls. THP-1 macrophage cell line (n = 1) and human umbilical vein endothelial cell line (HUVECs) (n = 1) were expanded in culture and then seeded onto each of the different disc preparations at a density of 12 x 10⁴ (HUVECs) and 16 x 10⁴ (THP-1) cells/disc respectively. Live/dead staining (day 1 and 5) and resazurin (Day 1, 3, 5 and 7) were performed for both cell types. Discs were fixed and subsequently immunostained for vascular markers (CD31) and macrophage markers (CD68 (M1) and CD163 (M2)).

Results: HUVECs exhibited greater cell viability and increased metabolic activity on LT-Ti discs than on SM-Ti discs. On CaSi₂-coated discs, HUVECs formed conglomerates on CaSi₂-emulsion coated SM-Ti discs but showed a homogeneous cell distribution and enhanced cellular viability on LT-Ti discs. CD-31 staining revealed vascular connections between HUVECs on LT-Ti and CaSi₂-coated LT-Ti discs. Macrophages were more viable and metabolically active on LT-Ti than SM-Ti discs with or without CaSi₂ coating. Immunostaining demonstrated that LT-Ti and CaSi₂ coated LT-Ti discs, had a higher proportion of CD-163 positive cells compared to corresponding SM-Ti discs.

Conclusion/significance: Our results show that novel LT-Ti discs coated with CaSi₂ supports the development of vascular structures and induces reparative macrophages that would contribute to implant integration. This would help prevent early or late implant loosening, thereby significantly improving implant longevity and performance within the patient.

Po1_1.21

The Role of Mineral Heterogeneity, Trabecular Orientation, and Collagen Fibers Arrangement on Tissue-Level Trabecular Bone Mechanics

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Abstract

Trabecular bone is a porous tissue, with a multiscale heterogeneous and anisotropic microstructure. At the architectural length scale, it features a complex network of beams (i.e. trabeculae). At the tissue level each trabecula comprises multiple bone packets, with different shapes, mineral content, and collagen arrangement [1]. The influence of material heterogeneity and structural anisotropy on the mechanical behaviour of trabecular bone remains poorly understood. Here, tissue-level mechanical properties of trabecular bone were considered in individual bone packets, in relation to local mineral content and collagen organization. A post-mortem trabecular bone sample was extracted from the iliac crest of a healthy 93 y.o. woman. Quantitative backscattered electron imaging (qBEI) generated 2-dimensional maps of the mineral content, while micro-computed tomography (micro-CT) provided 3D information on the trabecular network below the exposed surface. By superimposing the two techniques, trabeculae showing similar Ca content and oriented parallel and perpendicular to the qBEI plane were selected, to address microstructural anisotropy. Indentation elastic modulus was measured on the selected trabeculae using nanoindentation (nIND). Second harmonic generation microscopy (SHG) was employed to assess collagen organization qualitatively. Results from qBEI and nIND for all indents are shown in Fig. 1, demonstrating that for comparable values of mineral content, the measured elastic modulus tends to be higher for perpendicular trabeculae, which were in average 1.38 stiffer than horizontal trabeculae. SHG images (Fig. 2) reveal well-defined lamellar patterns in both trabeculae. Discontinuities at cement lines separating different bone packets were observed, with some neighbouring packets displaying differently oriented lamellae. Notably, SHG images of perpendicular trabeculae showed a weaker signal (darker grey levels) compared to parallel trabeculae, indicating a higher degree of out-of-plane fibril orientation, as SHG signals arise mainly from in-plane collagen fibril [2]. Thus, collagen fibrils are predominantly aligned along the trabecular axis, this is consistent with the mechanical anisotropy shown by nIND, as reported in literature [3]. These results will inform a computational model to unravel the interplay between trabecular microstructure and material heterogeneity in trabecular bone mechanics.

[1] Smith et al., Journal of Biomechanics 43, 2010; [2] Collins et al., Materialia 9, 2020; [3] Reznikov et al., Bone 71, 2015

Po1_1.22

Bridging Biology and Engineering: Pre-Vascularized Grafts for Craniofacial Therapy

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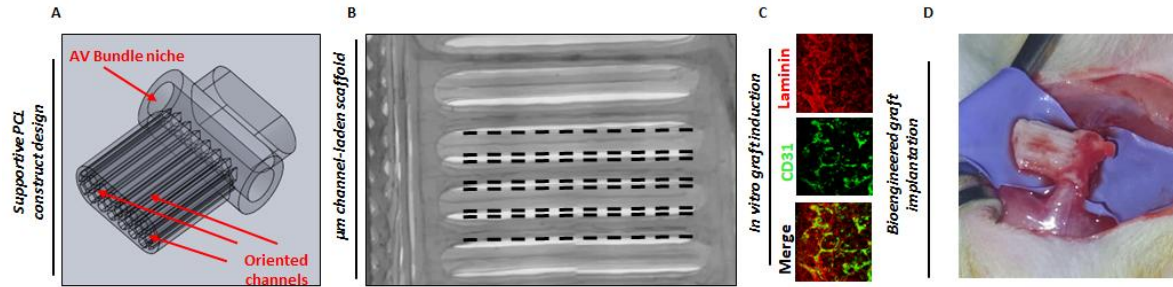
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Abstract

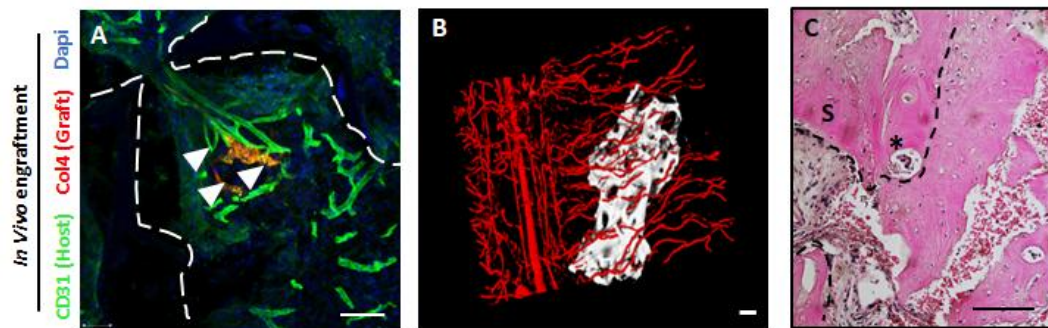
Over the past 25 years, oral squamous cell carcinoma (OSCC) has been on the rise, especially in young individuals without known risk factors, leading to over 300,000 new cases annually. Treatment often requires extensive resections of soft tissues and supporting bony framework, creating significant oro-facial defects with limited vascular support. Consequently, surgeons must perform substantial autologous grafting for defect reconstruction, with significant limitations due to the extent of bone vascularity, shortage of soft tissues, limited graft volume, and donor site morbidity. Maxillofacial surgeons and tissue engineers are challenged to create biologically inspired tissue replacements that reduce the need for patient tissue harvesting.

Recently, 3D-printed bone scaffolds have been explored for reconstructing significant defects, with various materials and deposition strategies replicating complex anatomies. A tissue-engineered approach has been used to fabricate human-scaled mandibular bone using a biomimetic extracellular matrix (ECM), leveraging the host's innate regenerative potential. However, the success of these constructs depends heavily on the host's capillary invasion into the graft, a slow process that often ends with graft necrosis.

We present a new approach combining 3D printing and tissue engineering to create highly vascularized tissue grafts for implantation. Polycaprolactone scaffolds were designed with channels to guide axial vessel formation. These scaffolds were populated with endothelial and stem cells to promote angiogenesis in vitro and in vivo. After implantation in an animal model, scaffolds facilitate guided vascularization and enrichment by the host's capillaries. A bone compartment is introduced within scaffolds containing an osteogenic matrix. The dual-compartment construct undergoes implantation to form composite neotissues. This translational study can potentially create grafts with vascularized and bone tissues that may provide a breakthrough toward an unmet clinical need: adding a reconstructive tool for rehabilitating patients undergoing reconstruction.



Experimental outline: A) Support structure with oriented channels (400 μm) and a predesigned niche for macro-vessels. (B) High resolution images of a 3D-printed construct with oriented channels. (C) Confocal Imaging of prevascularized scaffolds, exhibiting deposition of ECM fibers (Red) and apillary formation (green) (D) Bioengineered scaffolds implantation study.



Neotissue formation (A) 21 days after implantation host capillaries (Green) are visualized penetrating into scaffold pores (dashed lines), co-labeled vascular ECM components from original grafts (Red). (B) Bone matrix within neo-tissues. MicroCT depicts host vasculature penetrating into bone (C) H&E images of bone scaffolds depict erythrocytes-filled capillaries penetrating into bone scaffold compartments (asterisks). Scale bar=50 μm (A), 100 μm (B) 200 μm (C)

Po1_1.23

ATR inhibition reverses doxorubicin resistance in human osteosarcoma cells by increasing DNA damage response

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Abstract

Osteosarcoma is the most common primary tumor of bone, and doxorubicin is a frontline chemotherapeutic agent to treatment regimens. However, doxorubicin-associated multidrug resistance remains a significant obstacle to successful chemotherapy treatment for osteosarcoma patients. Our prior studies have shown that Ataxia-telangiectasia and Rad3 related protein kinase (ATR) expression and activation correlated with shorter patient survival and less necrosis following neoadjuvant chemotherapy in osteosarcoma. ATR inhibition suppressed cell proliferation in a time- and dose-dependent manner and induced apoptosis. In the current study, we further investigated the function and role of ATR in doxorubicin-resistant osteosarcoma cells. ATR-specific inhibitor VE-822 was applied to determine the effect of ATR inhibition on osteosarcoma cell growth and doxorubicin sensitivities. Expression of phosphorylated ATR in drug-resistant osteosarcoma cells were identified by immunofluorescence. Apoptosis and cell cycle were examined by Western blot and flow cytometry. The results showed ATR inhibition in doxorubicin-resistant osteosarcoma cells not only decreases cell growth and proliferation, but also significantly and synergistically enhances chemosensitivity to doxorubicin. In addition, combination treatment with VE-822 and doxorubicin reduced Chk1 phosphorylation while increasing γ H2AX expression and PARP cleavage, consistent with the increasing DNA damage responses. Flow cytometry analysis also demonstrated that ATR inhibition counteracts the G2/M phase blockade induced by DNA damage repair, enhances apoptosis and promotes cell death. Our findings support ATR as critical to osteosarcoma proliferation and doxorubicin resistance in osteosarcoma. Therefore, the addition of ATR inhibition to a standard doxorubicin regimen is a potential treatment strategy for osteosarcoma.

Po1_1.24

Regeneration of Critical-Size Bone Defects in a Rat Model Using Demineralized Bone Matrix and Low-Intensity Pulsed Ultrasound

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Abstract

Critical-sized bone defects pose a persistent therapeutic challenge in periodontology and maxillofacial surgery due to their limited self-healing capacity, highlighting the need for more efficient regenerative approaches. In clinical settings, free-packed bone graft systems are frequently employed due to their ease of application, though their regenerative efficacy diminishes in large or critical-sized defects. To address these limitations, adjunctive therapies such as low-intensity pulsed ultrasound (LIPUS)—a non-invasive modality shown to promote bone repair in orthopedics—have gained interest, although its potential remains largely unexplored in dental and craniofacial applications. Therefore, this study explores the combined application of LIPUS and a demineralized bone matrix (DIZG), used as an osteoinductive free-packed system, to assess their potential to enhance bone regeneration in critical-sized calvarial defects formed in rats.

Critical-sized calvarial defects were created in 11-week-old Wistar rats. The defects were filled with DIZG, while empty defects served as controls. LIPUS treatment on bone defect sites was applied for 15 days at an intensity of 30 mW/cm² for 20 minutes over a 3-week period. Eight weeks after initial defect formation, newly formed bone volume was assessed using micro-computed tomography, and histological analysis was performed to evaluate tissue organization and DIZG integration.

Results demonstrated that DIZG significantly enhanced new bone formation and successfully integrated with host tissue by 8 weeks post-implantation, supporting its osteoinductive capacity. LIPUS treatment for 3 weeks further promoted local bone formation by increasing osteogenic foci, particularly at the tissue-graft interface. However, this localized stimulation did not result in a statistically significant increase in regenerated bone volume. A similar effect was observed in empty defects, where LIPUS did not significantly increase bone volume but promoted the maturation of newly formed bone compared to unstimulated controls.

The lack of a significant increase in bone volume at 8 weeks post-defect formation—regardless of whether DIZG was used or the defect was left empty—suggests that 3 weeks of LIPUS stimulation did not substantially accelerate early-stage bone regeneration.

Nevertheless, LIPUS enhanced early osteogenic activity and promoted the newly formed bone maturation, particularly at the interface with the graft material. This suggest that the regenerative effects of LIPUS may become more pronounced at later stages, as the interplay between mechanical stimulation, the free-packed system, and the biological response progresses. Future studies should incorporate longer observation and/or longer stimulation periods to determine whether LIPUS can further augment bone regeneration in free-packed systems over time.

Po1_1.25

Adaptation of the mouse tibia to fatigue under weekly pathological *in vivo* axial loading

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Abstract

Stress fractures are not yet fully understood, but interest in this topic has increased due to the increasing prevalence of insufficiency fractures caused by habitual daily loading in the elderly. Stress fractures likely develop from the accumulation of microdamage caused by repetitive loading. Under normal circumstances, bone cells continuously renew bone; however estrogen deficiency, corticosteroids (CS) or bisphosphonates (BP) can disrupt this process. The study aimed to develop a murine model to investigate the response of bone to fatigue loading in the presence or absence of CS/BP.

84 female C57BL6 mice were divided into 6 groups. Groups 1 to 3 were ovariectomized at week 12 (W12). From W20 on, groups 2 and 3 received BP (zoledronic acid). Group 3 received a CS (Prednisolone). From W25 on, the left tibia in groups 1-4 were axially loaded (3-18N, 0.5Hz until 140% deformation or 3000 cycles). Group 4-5 were not loaded. Group 5 received analgesics only. The tibias were scanned bi-weekly with microCT. After sacrifice, tibias were tested in axial fatigue or used for histology.

The number of loading cycles until 140% deformation was very variable at W1 (1500±1000 cycles) but by W4 all groups had converged to 860±100 cycles (Fig. 1 A). Groups 3 and 4 exhibited the most notable increases in cortical thickness (ct.th) and bone volume (BV) at proximal tibia (Fig. 1B), reaching or surpassing the control levels. No significant changes were observed at the distal tibia.

Dynamic microCT imaging revealed that bone formation was greater in loaded compared to non-loaded tibias, while resorption showed only minor differences. Significant formation of woven bone was observed in the proximal region of the loaded tibias, and mechanical tests demonstrated that these tibias were significantly more resistant to fatigue than the non-loaded controls. Histological observation of microfractures and microcracks revealed that they occur very rarely.

Our data indicates that even with intense weekly *in vivo* fatigue loading, microdamage does not accumulate enough to significantly weaken mouse bones, regardless of estrogen levels, bisphosphonate or corticosteroid exposure. The absence of microdamage after fatigue loading was also confirmed by histological analyses. Even when fatigue loading exceeds traditionally accepted physiological limits in this model, it can still stimulate bone adaptation rather than causing weakening.

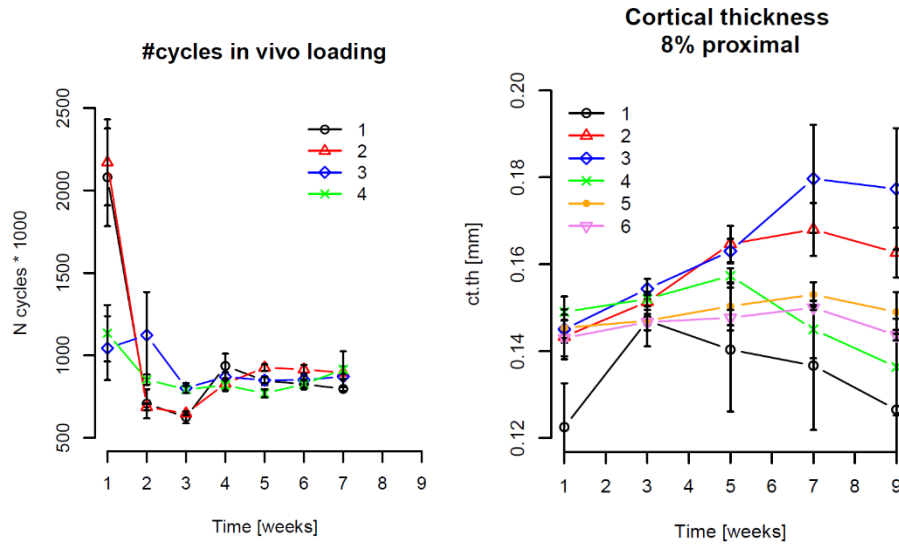


Fig. 1. Convergence of number of loading cycles (A) and evolution of ct.th in the proximal tibia (B).

Po1_1.29

Development of a Prototype Model for Lateral Split-Depressed Tibial Plateau for Bench Testing and Fixation Studies

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Abstract

Lateral split-depressed tibial plateau fractures (Schatzker Type II) (Fig. 1a) are the most common tibial plateau fractures and pose considerable challenges due to the combination of split fragments and articular depression, which impair load-bearing capacity. Developing a reliable bench-testing model is crucial for evaluating fixation strategies, optimising clinical outcomes, and reducing dependency on *in vivo* animal testing. This study presents a two-stage model development process including, a prototype fracture model using Sawbone (Fig. 1b) to simulate the fracture pattern, followed by the development and validation of the model in an *ex vivo* sheep tibia platform. This combined approach aimed to provide a robust and reproducible testing environment for evaluating fracture stabilization strategies and fixation methods while reducing reliance on *in vivo* testing.

The Sawbone (SAWBONES, USA) prototype replicated key features of Schatzker Type II fractures, including a standardised lateral osteoarticular defect (Ø10 mm, depth =7-10 mm), a controlled split of the lateral condyle, and an induced bone void to simulate compromised stability. Insights from the Sawbone model guided the preparation of an *ex-vivo* sheep tibia model to mimic clinically relevant conditions more accurately. Bone substitutes, including advanced adhesives with load-sharing properties and conventional non-adhesive void fillers, were injected into the defect and subjected to mechanical compression testing with a Zwick Z2.5 Testing Machine, simulating physiological loading conditions observed during weight-bearing activities. The *ex-vivo* sheep tibia model (Fig. 1c) effectively assessed the mechanical properties of bone substitutes and their ability to stabilise lateral split-depressed fractures. It also allowed for a detailed evaluation of failure mechanisms, such as angular failure and osteoarticular defect displacement, providing valuable insights into the stabilisation performance of different materials.

These models offer a reproducible, standardised, and ethical platform for investigating fracture stabilisation and fixation strategies under clinically relevant conditions. complex fracture patterns and loading scenarios, this approach can reduce dependency on *in vivo* animal testing while accelerating the development and translation of innovative materials and techniques into clinical practice. The prototype tibial models developed are essential for evaluating fracture stabilisation methods and bone adhesive performance in complex lateral split-depressed tibial plateau fractures, enhancing clinical applicability and advancing ethical preclinical research.

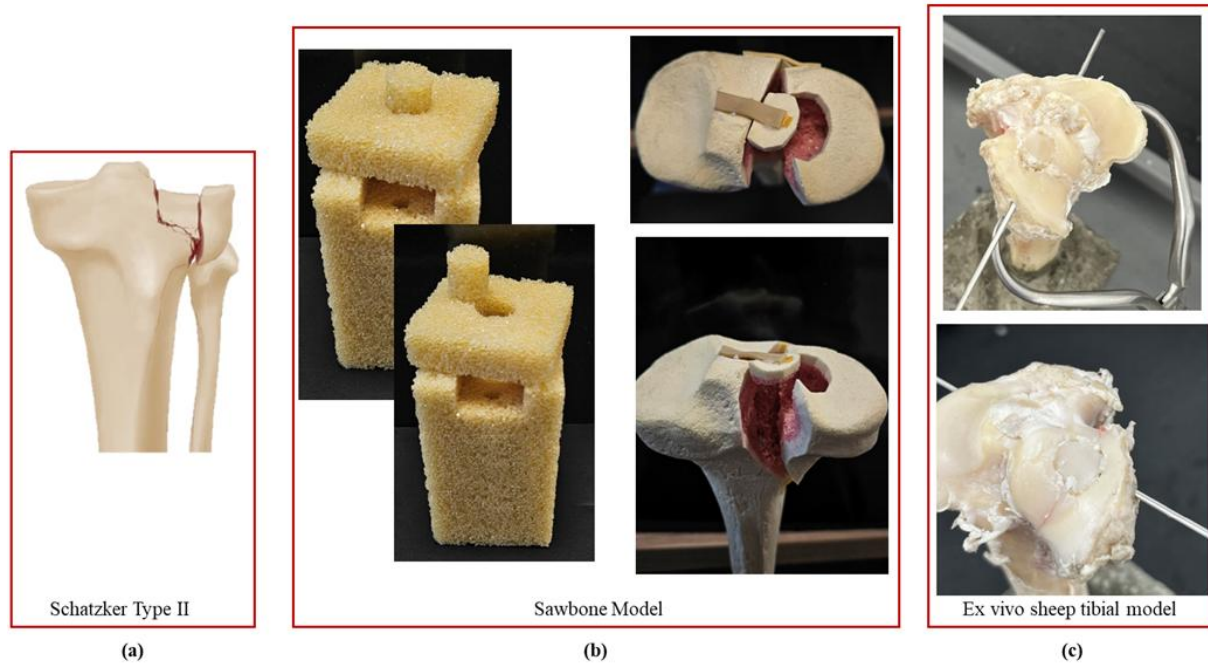


Fig. 1: Development stages of the lateral split-depressed tibial plateau fracture model: (a) Clinical representation, (b) Sawbone prototype with standardised fracture and defect, (c) Ex-vivo sheep tibia model replicating clinically relevant fracture patterns.

Po1_1.32

Hepatokine AGP2 Mediates Physical Exercise-Induced Bone Augmentation through Liver-Bone Signaling Communication.

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Abstract

Objective: Physical exercise has been demonstrated to be beneficial for bone health. However, the mechanisms behind physical exercise induced osteogenesis are still unclear, preventing us from developing “exercise mimetics” to resist osteoporosis. Emerging evidence underscores the liver as a central regulator in metabolic bone diseases, mediated by hepatokines. To investigate the role of AGP2, a hepatokine, in modulating bone density and strength through exercise-induced mechanisms, with a focus on how AGP2 regulates bone anabolism by modulating osteoblast and osteoclast activities in response to physical exercise.

Methods: We employed a multifaceted approach, including genetic manipulation of AGP2 expression in hepatocytes and biochemical assays to examine its function. Before and after exercise, we screened and evaluated AGP2 expression levels in human and mouse models through proteomic analysis. In mice, we utilized hepatocyte-specific deletion and liver-specific overexpression models to assess the effects of AGP2 on bone health. Using Cut-tag technology and base editing, we analyzed histone lactylation at H3K14 within hepatocytes to understand the regulatory mechanisms of AGP2 expression during exercise. Additionally, we used in vitro experiments to assess the impact of AGP2 on osteoblast mineralization and osteoclast activity through the Wnt/ β -catenin signaling pathway.

Results: Our results showed that exercise significantly elevated AGP2 levels in the serum of both human participants and mouse models. Hepatocyte-specific deletion of AGP2 eliminated the bone anabolic effects of exercise, even affecting the trajectory and thickness of trabecular bone and cortical bone. Meanwhile, liver-specific overexpression of AGP2 mitigated bone loss in ovariectomized mice, confirming AGP2's osteogenic potential. We found that exercise-induced AGP2 expression is regulated by histone lactylation at the H3K14 site, which is triggered by lactate accumulation during intense exercise. AGP2 activates the Wnt/ β -catenin signaling pathway through interaction with the LRP5 receptor on osteoblasts, promoting their mineralization and activity.

Conclusion: Our findings provide insights into the liver-bone axis, identifying AGP2 as a critical mediator of bone anabolism through exercise-induced metabolic signaling. These results highlight the potential of AGP2 as a therapeutic target for the treatment of metabolic bone diseases, particularly osteoporosis, by enhancing hepatokine signaling pathways. This work opens avenues for developing new strategies aimed at improving bone health through modulation of liver-derived factors.

Po1_1.34

Single-cell and spatial transcriptomics reveal cellular features and crosstalk between synovium and cartilage in human haemophilic arthritis

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Abstract

Unravelling the mechanism of molecular cross-talk between human cartilage and synovium could help unravel the key pathogenesis of haemophilic arthritis and provide therapeutic strategies. Here, we analysed the cellular features of synovium and cartilage in human osteoarthritis (OA) and haemophilic arthritis (HA) at the single-cell RNA sequencing (scRNA-seq) level, as well as the interactions in the pathogenesis. We profiled the transcriptomes of chondrocytes (53,287 cells) and synoviocytes (90,608 cells) isolated from OA and HA patients at a single-cell resolution. We first identified synovial and chondrocyte cell types in haemophilic arthritis and osteoarthritis. In addition, we identified abnormal molecular and cellular features of HA patients and cell-cell exchange communication in the knee environment. This study provides the basis for a panoramic cell atlas of hemophilia. Altered cell-cell communication between HA cell subtypes mediated through specific signalling pathways may be some of the important underlying mechanisms associated with the progression of haemophilic arthritis. It reflects the changes in synovial and cartilage interrelationships during haemophilia progression and suggests mechanisms that could serve as potential therapeutic targets.

Po1_2.03

The molecular path of PRP formulations on osteoarthritic chondrocytes presenting a tight therapeutic window

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Abstract

Osteoarthritis (OA) is a joint disease that occurs with a cycle of inflammation, degeneration and regeneration in articular cartilage, subchondral bone and surrounding tissues, known as the leading cause of disability among older populations (1). Platelet-rich plasma (PRP) attracts attention as one of the prominent biological treatment options in the treatment of OA, however, its composition and therapeutic efficacy and mechanism of action on OA remain unclear. Herein, we hypothesized that active PRP and PRGF might regulate autophagy, apoptosis and proliferation and provide regeneration at a tight therapeutic dose window in vitro. PRGF provided an increase in autophagic vacuoles in OA chondrocytes by immunofluorescence. Active PRP increased early and late apoptotic cell number in OA chondrocytes compared to healthy chondrocytes, whereas PRGF reduced the rate of early apoptotic cells and induced late apoptosis compared to control and healthy chondrocytes by flow cytometry. Both PRPs promoted collagen II and aggrecan labelings by immunofluorescence and TGF- β and PDGF-BB expressions by ELISA. PRPs might serve as a cutting-edge approach for diagnosis and treatment of OA in the clinic at a tight therapeutic window.

Keywords: Osteoarthritis (OA), Platelet-rich plasma (PRP), Autophagy, Proliferation, Apoptosis, Regeneration, Chondrocyte

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Acknowledgements

This research was supported within the scope of TÜBİTAK 1001-Scientific and Technological Research Projects Support Program (#222S093).

Po1_2.04

Chondrocyte mitochondrial dysfunction as a target for osteoarthritis therapy: identification of a new molecule

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Abstract

Introduction: Osteoarthritis (OA) is the most prevalent joint disease leading to cartilage degradation, subchondral bone remodelling, and synovial inflammation. However, no disease-modifying drugs are currently available. Mitochondrial dysfunction, including defective oxidative phosphorylation (OxPhos) and favoured glycolysis, is observed in OA. Therefore, we aim to develop mitochondria-targeted strategies by exploring molecules that enhance mitochondrial bioenergetics in other diseases. First, we compared the structure of mitochondria between normal and OA cartilage. Secondly, we screened molecules for their ability to improve or restore OxPhos function in OA chondrocytes.

Method: Cartilage samples were obtained from the facet joint of scoliotic patients and the knee joints of patients with end-stage OA. To minimize age and anatomical site biases, matched samples were collected from both undamaged and damaged areas of OA cartilage. The samples were prepared for transmission electron microscopy (TEM), allowing the characterisation of mitochondria in terms of number, size, and morphology using QuPath software. Mitochondrial DNA (mtDNA) was extracted to determine copy number. To assess bioenergetics function, ATP rate and mitostress assays were performed on chondrocytes isolated from normal (NC) and OA cartilage (OAC) using Seahorse. L-BAIBA, nobiletin, and bezafibrate (0.1-10 μ M for 24h) were screened for their potential to modulate OxPhos activity in OAC.

Result: TEM analysis indicates an increased number of mitochondria in OA cartilage compared to normal cartilage (N=3, 10 cells/sample), which was supported by a significant increase in mtDNA copy number (N=5 per group, $p=0.0317$). A similar increase in mitochondrial number is observed by TEM in the damaged zones compared to the undamaged OA zones (N=3, 10 cells/sample). Mitochondrial morphology appears unchanged in all groups. In vitro, ATP rate assays reveal a reduced reliance on OxPhos for OAC (24%) compared to NC (40%) (N=4, N=2). Among the molecules tested, bezafibrate (1 μ M for 24h) tends to improve maximal respiration and ATP production in OAC (N=4, $p=0.0571$).

Conclusion: Our results suggest that OA cartilage exhibits an increased number of mitochondria. However, inclusion of additional cartilage samples are required to confirm these observations. This potential increase in mitochondrial number appears to be associated with a reduction in OxPhos activity in OAC compared to NC. Bezafibrate, a peroxisome proliferator-activated receptor (PPAR) agonist, shows promise in enhancing OxPhos function in OAC. Nevertheless, we still need to ascertain whether bezafibrate can boost ATP production by improving OxPhos activity, before considering its therapeutic potential for OA.

Po1_2.08

Novel approach to identify disease severity marker candidates in osteoarthritis patients based on gene expression changes in human chondral plugs and their association with electromechanical properties as a proxy for cartilage pathology

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Abstract

Traditionally, cartilage transcriptome analysis in OA-patients is performed on chondrocytes harvested from the outer lateral tibia plateau (less damaged area), for comparison with material taken from the inner medial, highly damaged area. The classical approach with visual selection does not allow harvesting samples based on objective descriptors of cartilage damage. As an alternative, we investigated the possibility to identify molecular signatures that are associated with OA-damage using electromechanical classification by ArthroBSTTM (Biomomentum Inc) to establish a QP-score as a proxy of cartilage damage. Genes were further filtered based on detection in synovial fluid and plasma proteome analysis using SomaScan® (Somalogic Inc).

Hypothesis: The identification of cartilage damage associated transcripts using QP-score will improve our understanding of OA-pathologies and facilitate the discovery of potential novel disease severity plasma marker candidates.

A prospective cohort study was conducted in 119 patients with end-stage knee OA scheduled for total knee arthroplasty. The tibia was collected and ex vivo electromechanical properties of the tibial cartilage, as a proxy for cartilage damage, were evaluated using ArthroBSTTM. The device measures streaming potentials generated during a rapid compression of the articular cartilage with an array of microelectrodes lying on a semi-spherical indenter, and calculates a quantitative parameter (QP, arbitrary units), generating a heat map of cartilage damage across the tibia plateau. Guided by this heat map, we collected 10 cartilage cores/patients (total of 1200 cores) from the tibia plateau across the full range of pathologies with matched QP for RNAseq analysis.

Results: We identified 31 genes positively associated with cartilage damage including SERPINE1, CRTAC1, TNFSF15, GAS1, TNFRSF11B, TREM1, INHBA, DKK3, IL11, CXCL14, NPTX2, FGF9. A negative association with cartilage damage was found with 25 genes, including MMP10, FRZB, BST2, LAMA4, FBLN1, IBSP. Adding synovial fluid proteomics to our analysis, we identified 25 unique candidate genes that decrease with cartilage damage (QP)

and associate with synovial fluid proteins decreasing in abundance with KL-grade. Several recently identified serum biomarkers predictive of OA (Kraus et al 2024) overlap with the cartilage damage genes identified in our study, including upregulated CRTAC1, COMP, APOB and negatively regulated RBP4 and FBLN1.

Conclusion: Determination of electromechanical properties of OA-cartilage is a novel, promising method to guide transcriptome analysis and its association with OA-pathology

Po1_2.09

Characterisation of Cultured Human Chondrocytes Conditioned Under Low Oxygen

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Abstract

In regenerative medicine, therapies like autologous chondrocyte implantation (ACI) can treat chondral defects. ACI provides potentially better results than microfracture but is ultimately constrained by specific indications and longer-term outcomes. Changing cell culture conditions during manufacture may be of clinical relevance. Here, we explore the hypothesis that a chondrocyte culture using a hypoxic chamber might enhance its function by improving extracellular matrix (ECM) production and modulating the immune environment.

We isolated human articular chondrocytes (HAC) from cartilage obtained at joint replacement surgery and cultured them until 80% confluent in standard oxygen conditions (21%). The cells were then divided and cultured under low-oxygen (2%) or standard-oxygen conditions. We then conducted ATP/lactate assays, RT-PCR, and immunocytochemistry (ICC) to assess the specific protein presence under varying oxygen levels.

Short exposure of HAC to hypoxia increases the expression of chondrogenic genes *COL2A1*, *SOX9*, *ACAN*, and, to some extent, *COMP*. *COL2A1* and *ACAN* expression was also confirmed at the translational level using immunocytochemistry (ICC). While it is well-documented that hypoxia elevates *COL2A1* expression, this effect is evident after a short exposure period of 48 hours.

RT-qPCR results indicated decreased expression of the inflammatory-associated genes *TNF* and *IL1B* under hypoxic conditions. In contrast, *RUNX2* expression increased, along with *IL6* levels. However, hypoxia inhibited the translation of *RUNX2* at the protein level, as cytoplasmic *RUNX2* expression was not detected at low oxygen levels (2% hypoxia).

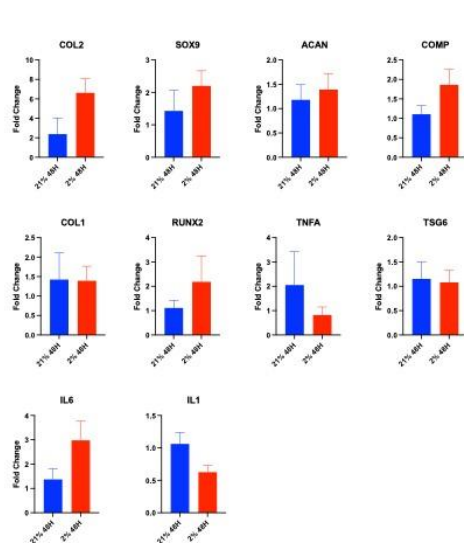
The increased abundance of *RUNX2* transcripts was not mirrored by protein levels in the cytoplasm. However, the *RUNX* protein localised in the nucleus was the same across both conditions.

There was no significant change in *TNFAIP6* levels in either ICC or RT-PCR. The uniform localisation of protein across the cells highlights their rounded morphology, resembling hyaline chondrocytes embedded in cartilage under hypoxic conditions.

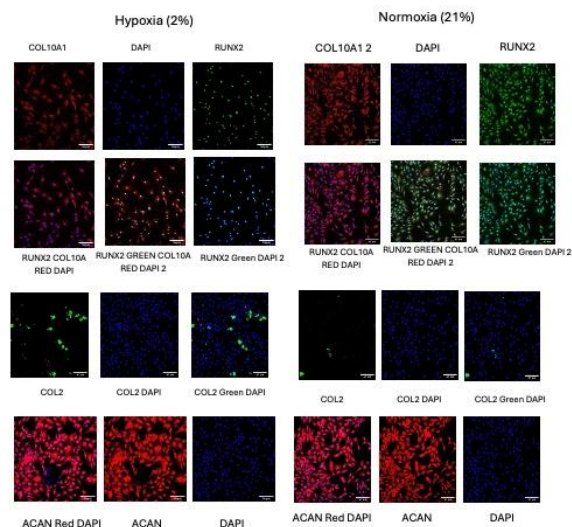
Hypoxic conditions showed a denser *ACAN* pattern in ICC compared to normoxia. Additionally, increased levels of acetylated microtubules were observed in hypoxic

environments, where tubulin acetylation is believed to boost structural rigidity. The altered cytoskeletal structure may prepare cells to protect against or respond to mechanical stress. Metabolic differences in the ratio of ATP to lactate between chondrocytes cultured in normoxia versus hypoxia were observed.

In conclusion, short exposure to hypoxia promotes the expression of the matrix components' deposition markers, potentially enhancing chondrocyte structural integrity. Single-cell transcriptomics is underway to understand the heterogeneity of chondrocyte populations exposed to hypoxia and whether selected populations can improve chondrocyte matrix production.



Rt-PCR results show that short exposure of primary human chondrocytes to hypoxia increases the expression of chondrogenic genes COL2A1, SOX9, ACAN, and, to some extent, COMP. COL2A1 and ACAN expression



ICC results show that short exposure of primary human chondrocytes to hypoxia caused an increased abundance of RUNX2 transcripts that was not mirrored by protein levels in the cytoplasm. However, the RUNX protein localised in the nucleus was the same across both conditions. ACAN had a denser pattern in hypoxia

Po1_2.10

Chemical Induction and Regulation of Functional Cartilage Repair

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Abstract

Chemical reprogramming provides an unprecedented opportunity to control somatic cell fate and generate desired cell types, including pluripotent stem cells, in a precise, flexible, and controllable manner for biomedical applications. Studies have shown that human somatic cells can successfully acquire pluripotency through chemical reprogramming, enabling the control of cell fate progression. Chemical small molecules target specific drug targets, promoting chondrocyte differentiation and extracellular matrix (ECM) production while inhibiting inflammatory mediators, thereby facilitating the repair of osteoarthritic (OA) articular cartilage. By enhancing the transition of chondrocytes to a more adaptive state, chemical small molecules increase their regenerative potential and ability to maintain cellular homeostasis in dynamic environments. Cinnamyl alcohol (CA), a small molecule compound extracted from cinnamon, has been shown to exert anti-inflammatory effects through the NLRP3 inflammasome pathway. This study aims to investigate the therapeutic effects of CA on osteoarthritis (OA) and its underlying molecular mechanisms of regulation. CA promotes the expression of anabolic markers COL2 and ACAN, as well as anti-inflammatory factors IL-4 and IL-10, while inhibiting the expression of pro-inflammatory factors IL-6 and TNF- α in C28/I2 cells. In LPS-induced RAW264.7 and THP-1 cells, CA suppresses IL-1 β , IL-6, and TNF- α levels and enhances IL-4 and IL-10 expression. In vivo, intra-articular injection of CA after DMM surgery promotes cartilage anabolism, reduces inflammation levels, and alleviates OA-associated pain. DARTS and mass spectrometry analyses identified PIGR and its domain 1 as the specific binding targets and major domains for CA. In chondrocytes, PIGR knockout attenuated the regulatory effects of CA on COL2, IL-4, and IL-10, which could be partially restored by re-expression of PIGR. Furthermore, in PIGR-knockout chondrocytes, the inhibitory effects of CA on YAP and NF- κ B phosphorylation were diminished, while re-expression of PIGR partially restored these inhibitory effects. In conclusion, CA exhibits effects in promoting cartilage anabolism, inhibiting pro-inflammatory factors, enhancing anti-inflammatory factors, repairing knee joint damage in DMM mice, and alleviating OA pain. Mechanistically, CA specifically binds to the first domain of PIGR and regulates the YAP/NF- κ B pathway, thereby mitigating inflammatory damage to the joints. These findings provide theoretical and experimental evidence supporting the clinical application of CA in treating OA.

Po1_2.11

POSTN potentiates chondrocyte hypertrophy by promoting RUNX2 in osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common degenerative disease with progressive cartilage loss as the primary pathogenesis. Chondrocyte hypertrophy, governed by the highly expressed Runt-related transcription factor 2 (RUNX2) and type X collagen gene (COL10A1), plays a pivotal role in the loss of chondrocyte function and cartilage degeneration. However, the molecular mechanisms underlying chondrocyte hypertrophy are still largely unknown. We conducted a comprehensive analysis of the transcriptome and proteome data obtained from human cartilage, leading to the identification of 49 differentially expressed genes. Subsequently, we performed correlation analysis between these genes and indicators associated with chondrocyte hypertrophy, revealing periostin (POSTN) as a potential regulator in this process. In this work, we investigated the role of POSTN in chondrocyte hypertrophy and its involvement in the pathogenesis of osteoarthritis. Our research revealed an upregulation of POSTN expression and an increase in chondrocyte hypertrophy in both human and mouse cartilage. In the mouse OA model, intra-articular injection of lentivirus targeting downregulation of POSTN significantly impeded the progression of osteoarthritis, including cartilage damage, synovial inflammation, joint swelling, and osteophyte formation. Upregulation of POSTN decreased the population of hypertrophic chondrocytes. Furthermore, mechanistic analysis revealed that the downregulation of POSTN can lead to a reduction in the expression of the key transcription factor RUNX2, which is responsible for promoting chondrocyte hypertrophy differentiation. Consequently, this ultimately results in a decrease in the expression of downstream genes related to hypertrophy that are regulated by RUNX2, thereby effectively inhibiting chondrocyte hypertrophy. In summary, our research unveils the role of POSTN in facilitating chondrocyte hypertrophy, positioning it as a potential therapeutic target for the alleviation of OA progression.

Po1_2.12

Outcomes of Meniscal Allograft Transplants: The Oswestry Experience

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Abstract

Purpose. Partial or complete loss of the meniscus leads to greater load distribution leading to the potential of increased cartilage damage. Meniscal allograft transplantation (MAT) is a viable option to reduce the associated risks in patients with symptomatic deficient menisci. The aim of this study is to report the outcomes of our MATs, at a tertiary centre, throughout the last 10 years.

Methods. Consecutive MATs done at a tertiary centre – The Robert Jones & Agnes Hunt Orthopaedic NHS Hospital were identified. Patient demographics, operative techniques used, concomitant surgical procedures, length of stay, patient reported outcomes including (Lysholm scores), graft characteristics, follow-up and complications were recorded and analysed. A post-operative Lysholm score < 65 was regarded as a clinical failure.

Results. A total of 56 MATs were performed between 2015-2024 with a mean patient age at the time of surgery of 31 yrs (range 17 – 46). 32% were females and 68% were males with a mean Charlson Index of 0. 48% and 52% were done on the right and left knee respectively with 48% to replace the medial meniscus and 52% to replace the lateral meniscus. 31 patients (55%) have undergone additional procedures at the time of surgery – 23% Osteotomy, 32% ligament reconstruction, 58% cartilage regeneration and 6% a combination of the aforementioned. The mean length of stay (LOS) was 1 day (range 0 – 6), with 33% day case operations. 82% of the MATs were meniscus only and 18% with a bony block with a mean allograft donor age of 29 yrs (range 14 – 45). Lysholm Score improved by a mean of 30.2 points (range -26 – +71). 30% had subsequent surgery to the same knee post-operatively with a 20% Graft failure rate. 13% had a MAT tear and 4% developed symptomatic arthritis which required a surgical intervention (osteotomy). The rate of infection, DVT, CRPS and stiffness was 2%, 2%, 2% and 7%, respectively.

Conclusion. MAT is an effective treatment for meniscal deficient knees that improves function and alleviates pain. However, there is a learning curve to the operative procedure and associated complications that should be highlighted during pre-operative discussions.

Po1_2.13

Learning Curve for Meniscal Allograft Transplants

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Abstract

Purpose. Meniscal allograft transplantation (MAT) is a viable option to improve function and pain in patients with symptomatic deficient menisci. However, as with many other orthopaedic procedures, there is a learning curve which has not been defined in the literature. Therefore, the aim of this study is to determine the surgical team's MATs learning curve, using our data throughout the last 10 years.

Methods. A database of MATs done at a tertiary centre – The Robert Jones & Agnes Hunt Orthopaedic NHS Hospital, was used to identify all operations performed from inception. A post-operative Lysholm score < 65 was regarded as a clinical failure. Surrogate measures of the learning curve including tourniquet times (TT), MAT failure, subsequent surgery on the same knee (SS), length of stay (LOS), PROMs and post-operative complications, were analysed.

Results. A total of 56 MATs in 55 patients were performed between 2015-2024. The MAT procedure was associated with a learning curve of 6 cases for tourniquet time, MAT failure and SS ($p < 0.05$). When comparing the first 6 cases (Group A) to the subsequent 50 cases (Group B), the mean tourniquet time was 116 mins as compared to 96 mins with a mean LOS of 2 vs. 1 day(s), respectively. The mean no. of SS was 1.5 (Range 0 – 3) vs. 0.3 (Range 0 – 2). 33% had a MAT tear and arthritis requiring a surgical intervention as compared to 12% and 0%, respectively. There was 1 case of infection in Group A vs. none in Group B. Group A had a 67% graft failure rate vs. 16% in group B. The mean postoperative Lysholm score for Group A was 54 vs. 75 for Group B with a mean difference between the pre-op and post-op PROMs of 21 and 29, respectively.

Conclusion. The MAT procedure was associated with a learning curve of 6 cases for tourniquet time, MAT failure and SS. This should be taken into consideration for soft tissue knee surgeons embarking on having MAT procedures as part of their operative portfolio.

Po1_2.14

Meniscal Allograft Transplants: Oversizing the grafts in the AP dimension Increases Post-operative Complications but Does Not Affect Failure Rates.

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Abstract

Purpose. Undersizing the meniscus graft in meniscal allograft transplants (MATs) has been reported to negatively affect the outcomes and failure rates but no studies looked at the effect of oversizing MATs on clinical outcomes. The aim of this study is to assess whether oversizing the MATs affects the complication and failure rates post-operatively.

Methods. A database of MATs done at a tertiary centre – The Robert Jones & Agnes Hunt Orthopaedic NHS Hospital, was checked to identify all operations performed from inception. Patient demographics, operative techniques used, concomitant surgical procedures, length of stay, patient reported outcomes (Lysholm scores), graft characteristics and dimensions using the MRI method, follow-up and complications were recorded and analysed. A post-operative Lysholm score < 65 was regarded as a clinical failure.

Results. A total of 56 MATs in 55 patients were performed between 2015-2024 with a mean age of 31 yrs (range 17 – 46). 48% and 52% were done to replace the medial meniscus and the lateral meniscus, respectively. 29 (58%) MATs were oversized in AP dimensions by a mean of 2.64 mm (Range 0.2 – 13.3). Of which 12 (41%) were Oversized by > 2 – 5mm and 3 (10%) by > 5mm. Oversizing the grafts in length, particularly > 2 – 5mm, were associated with an increased rate of complications with 17% MAT tears ($p = 0.03$), but not when the grafts were oversized in width. Oversizing the grafts was not associated with increased rate of clinical or mechanical failure in the post-operative period.

Conclusion. Accepting an allograft that is >2-5 mm larger in length (AP) than pre-op templating increases the likelihood of post-operative complications particularly related to MAT tears. Efforts should be made by the surgeons and their individual tissue bank provider to avoid graft-host mismatch that could affect outcome.

Po1_2.15

The choice of fixation device or the number of meniscus sutures used does not affect the post-operative complication rates nor patient reported outcomes following Meniscal Allograft Transplants

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Abstract

Purpose. The aim of this study is to investigate whether the number or type of meniscal sutures used or fixation device for securing the allograft in place affect the outcomes of our MATs, at a tertiary centre, throughout the last 10 years.

Methods. A database of MATs done at a tertiary centre – The Robert Jones & Agnes Hunt Orthopaedic NHS Hospital, was checked to identify all operations performed from inception. Patient demographics, operative techniques used, no. and type of meniscal sutures used, Lysholm scores, subsequent surgery on the same knee (SS) and complications including stiffness, failure and MAT tears were recorded and analysed. A post-operative Lysholm score < 65 was regarded as a clinical failure.

Results. A total of 56 MATs in 55 patients were performed between 2015-2024 with a mean patient age at the time of surgery of 31 yrs (range 17 – 46). 48% and 52% were done to replace the medial and lateral meniscus, respectively. 82% were meniscus allograft only and 18% with a bone block. The majority of MATs were fixed using a BTB tightrope for the posterior horn (71%) and Bioswivelock (36%) for the anterior horn. Multiple other fixation devices were used. Type of fixation device did not affect complication, failure, stiffness and MAT tear rates nor need for SS ($p > 0.05$). The median no. of sutures used was 8 (range 5 – 12) with a median of 5 for all inside, 3 for inside out and 1 for outside in sutures. Suture types or no. used did not influence complication, failure, stiffness and MAT tear rates nor need for SS ($p > 0.05$). Additionally, the fixation device and the sutures no. or type used were weakly correlated with the post-operative Lysholm scores ($p > 0.05$).

Conclusion. Neither the fixation device nor the number and type of meniscus sutures used influence the rate of post-operative complications including failure, stiffness and tears, or patient reported outcomes following MATs. This expands the choice of fixation devices to be used and provides re-assurance to the surgeon when it comes to fixing and securing the MAT.

Po1_2.16

Allograft Donor Age and Gender Does not Influence Post-operative Outcomes in Meniscal Allograft Transplant Surgery

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Abstract

Purpose

Several prognostic factors for meniscal allograft transplantation (MAT) has been described in the literature but none on graft donor age and gender. The aim of this study is to determine the effect of allograft donor age and gender on the outcomes of our MATs, at a tertiary centre, throughout the last 10 years.

Methods

A database of MATs done at a tertiary centre – The Robert Jones & Agnes Hunt Orthopaedic NHS Hospital, was checked to identify all operations performed from inception. Patient demographics, operative techniques used, Lysholm scores, graft characteristics including donor age and gender, subsequent surgery on the same knee (SS) and complications were recorded and analysed. A post-operative Lysholm score < 65 was regarded as a clinical failure. Patients with incomplete graft data were excluded.

Results

A total of 56 MATs were performed between 2015-2024 with 50 having complete graft data. The median allograft donor age was 28 yrs (Range 14 – 45 yrs) with 34% and 66% from male and female donors, respectively. Allograft Donor gender was not associated with increased rate of complications ($p = 0.1$), SS ($p = 0.21$) or MAT failure ($p = 0.52$). A post-hoc analysis using the Bonferroni's method confirms the aforementioned $X^2 = 7.98$. No Allografts donors were aged > 45 yrs. Allograft donor age was not associated with increased rate of complications ($p = 0.59$), SS ($p = 0.61$) or MAT failure ($p = 0.38$).

Conclusion

Rate of MAT complications, SS or failure was not dependent nor associated with the allograft donor age or gender. As long as the donor is < 45 yrs of age, this critical information expands the selection criteria and provides re-assurance to the surgeon when it comes tissue selection from the allograft provider in MAT procedures.

Po1_2.17

MRI-Based Assessment of Donor Site Healing Following OATS: Evidence of Good Cartilage Regeneration At a mean Follow-up of 1.9 Years

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Abstract

Introduction

Healing of the donor site following Osteochondral Autograft Transfer Surgery (OATS) has not been evaluated. Magnetic Resonance Imaging (MRI) offers a promising method for assessing donor site recovery and cartilage regeneration following OATS. This study aims to evaluate donor site cartilage regeneration following OATS using MRI.

Materials & Methods

A database was used to identify OATS cases from a tertiary centre where patients had a post-operative MRI for various reasons. Patients who had additional cartilage regeneration procedures performed to the donor site were excluded. Patient demographics, operative techniques, donor and recipient sites were evaluated. The International Cartilage Society (ICRS) grading was used to classify the defects and a Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) 2.0 system was applied to determine the quality of cartilage regeneration at the donor site on the post-operative MR images by two independent assessors.

Results

14 patients (mean age of 34 yrs.) met the inclusion criteria. The defects were located in the patella (36%), lateral femoral condyle (29%) and medial femoral condyle (36%) with a mean ICRS grade of 4. The mean time from surgery to the post-operative MRI was 1.9 yrs. (range 0.4 to 7.1 yrs.). 50% were performed on the left knee with a mean of 1 additional procedure performed at the time of index surgery. 43% and 48% had 8 mm and 10 mm plugs taken respectively. Donor sites included lateral (52%), central (29%) and medial (19%) trochlea. The median MOCART 2.0 score for all donor sites was 70 (range 35 – 90) with excellent inter-rater reliability (ICC=0.86).

Conclusion

Our findings show good robust donor site healing and effective cartilage regeneration following OATS, evidenced by MRI at a mean follow-up of 1.9 years. This provides reassurance regarding the reliability of OATS at the donor site when treating cartilage defects.

Po1_2.21

Causal relationship between hallux valgus, flat feet, and osteoarthritis: a bi-directional two-sample mendelian randomized study

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Abstract

Objectives: Previous research has highlighted a notable prevalence of Hallux Valgus (HV) and Flat Foot (FF) among Osteoarthritis (OA) patients. Yet, the nature of the causal relationship among these conditions remains ambiguous. This study aims to utilize Mendelian Randomization (MR) to explore and substantiate the potential causal links between HV, FF and OA.

Methods: A bi-directional two-sample MR was adopted to research the causal relationship between HV, FF and OA. The inverse variance weighted, MR-Egger and weighted median estimator methods were used to estimate bi-directional causal effects. Statistical analyses were performed using R software version 4.3.1.

Results: HV had a causal effect on six types of OA (all OA: OR:1.10, $P=4.53e-06$; hip OA: OR:1.13, $P=2.15e-03$; knee OA: OR:1.17, $P=5.84e-05$; hand OA: OR:1.17, $P=9.94e-05$; finger OA: OR:1.14, $P=1.53e-3$; thumb OA: OR:1.24, $P=9.48e-5$) and FF (OR:1.89, $P=1.54e-14$); Additionally, all OA or knee OA had a significant causal effect on both HV and FF (all OA-HV: OR:2.51, $P=1.13e-05$; all OA- FF: OR:2.72, $P=1.07e-03$; knee OA-HV: OR:1.64, $P=1.73e-7$; knee OA-FF: OR:1.86, $P=2.70e-5$); Furthermore, FF had a causal effect on HV (OR:1.42, $P=6.14e-6$).

Conclusions: Our findings indicate a causal association between HV, FF and OA. Specifically, individuals with knee OA exhibit a genetic predisposition to both HV and FF. Moreover, a causal effect of HV on the development of knee OA has been identified. Ultimately, our study uncovers a bidirectional causal association between FF and HV.

Po1_2.22

Serum biochemical markers and rheumatoid arthritis: Insights from observational study and Mendelian randomization analysis

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Abstract

Background: Serum biochemical markers, including albumin and globulin, are associated with the inflammatory aging process in individuals with rheumatoid arthritis (RA), reflecting its utility in monitoring disease progression and therapeutic responses. This study evaluated the impact of biochemical markers on RA and assessed their causal relationship.

Methods: Adults (≥ 18 years) with RA were identified from the 2001-2020 National Health and Nutrition Examination Survey (NHANES). Weighted logistic regression was used to evaluate the association between serum biochemical markers and RA. Restricted cubic spline (RCS) regression was conducted to explore non-linear associations between variables. Then, two-sample Mendelian randomization (MR) and Generalized summary data-based Mendelian randomization (GSMR) analysis were employed to analyze the causal relationships between biomarkers exhibiting differential expression identified within the NHANES database. We further performed replication and meta-analysis to enhance the confidence of causal associations.

Results: The NHANES cohort included 1,416 participants with clinically diagnosed RA and 3,744 control participants without RA. In the multivariable regression, albumin, calcium, globulin, lactate dehydrogenase (LDH), and uric acid levels were significantly associated with the prevalence of RA. A four-node RCS model demonstrated non-linear correlations between albumin, uric acid, and RA (all P non-linear < 0.05), while calcium, globulin, and LDH exhibited linear relationships (all P non-linear > 0.05). GSMR analysis revealed a significant causal relationship between serum albumin and RA risk (OR = 0.80, 95% CI: 0.72-0.89, $P = 3.67 \times 10^{-5}$). The IVW meta-analysis also confirmed that decreased albumin expression significantly associated with decreased risk of RA ($P = 0.002$).

Conclusion: This study found a linear association between calcium, globulin, LDH, and RA risk, while a U-shaped non-linear relationship between albumin, uric and RA was observed. MR analysis revealed a significant causal association between albumin and RA. However, the molecular mechanisms by which albumin affects the risk of RA remain to be further elucidated.

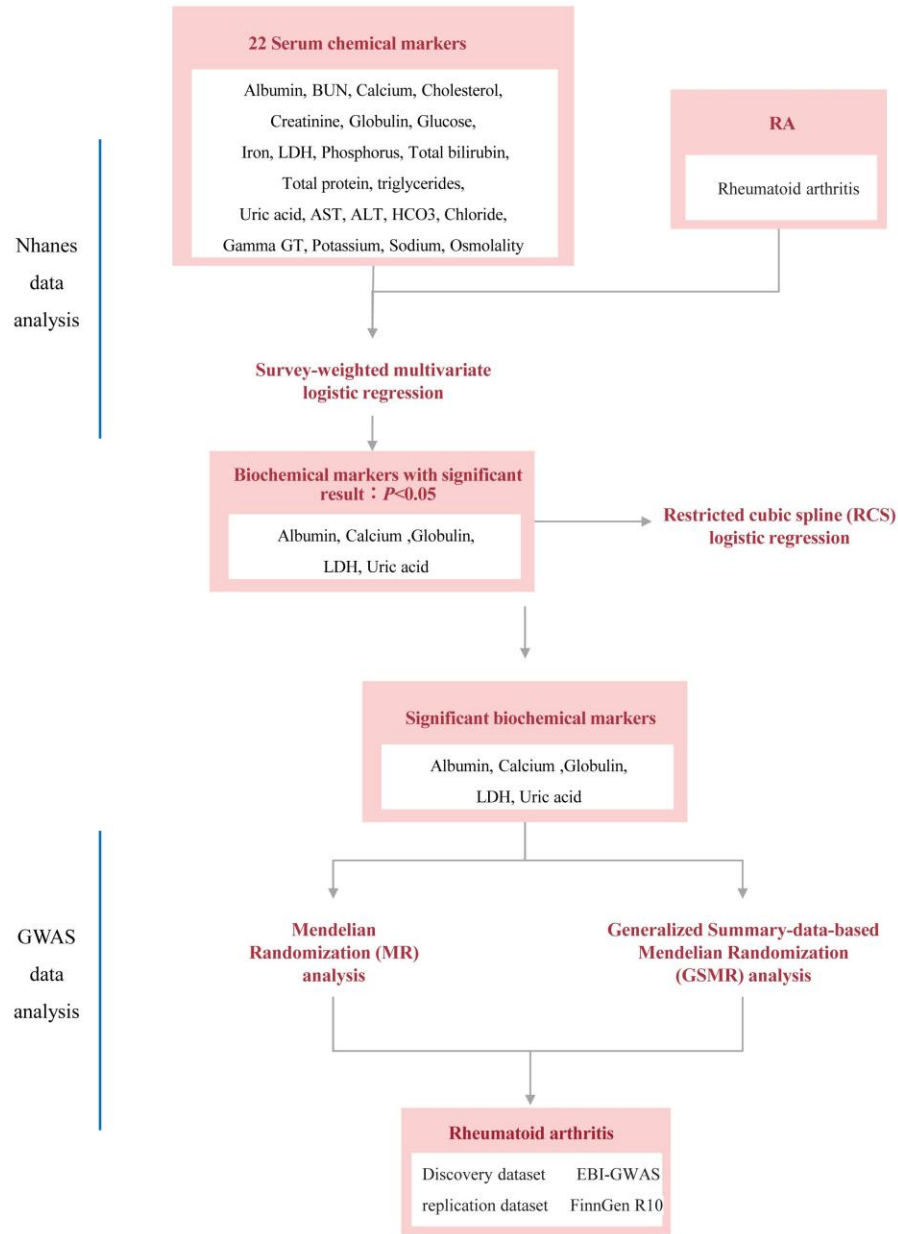


Fig.1 Outline of the study design

Po1_2.23

A Multi-Omics Journey into Synovial Fluid: Advancing Molecular Classification of Knee Osteoarthritis

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Abstract

This study advances the understanding and classification of osteoarthritis (OA) subtypes through integrated proteomic and metabolomic analyses of synovial fluid from 100 OA patients. Utilizing advanced unsupervised clustering, four distinct OA subtypes were identified: Bone Remodeling (C1), Pain-related (C2), Inflammation-driven (C3), and Cartilage Degradation (C4). Subtype C3 was characterized by significant neutrophil enrichment and a strong association with inflammatory biomarkers such as erythrocyte sedimentation rate and C-reactive protein. Furthermore, a pronounced crosstalk between subtypes C2 and C3 suggests that immune-driven mechanisms, particularly involving neutrophils, may enhance bone remodeling. Metabolomic profiling linked subtype C4 to oxidative stress and ferroptosis, elucidating specific pathways involved in cartilage degradation. Validation of the identified subtypes was performed using immunohistochemistry and Western blot analyses on human tissue samples, confirming the reliability of the classifications. These findings highlight the molecular heterogeneity among knee OA patients and provide a robust foundation for precision medicine approaches, facilitating the development of targeted therapeutic strategies tailored to specific OA subtypes to improve patient outcomes.

Po1_2.24

High osmolality mediates the Nfat5-Gas1-SHH axis to delay osteoarthritis

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Abstract

Osteoarthritis (OA) is currently the most common chronic degenerative disease and has become the fourth most common residual disease worldwide. Patients with OA usually present with joint swelling due to changes in synovial fluid within the joint. As one of the important physical properties of synovial fluid, osmotic pressure plays a decisive role in the homeostasis of the intra-articular microenvironment. Studies have shown that flushing with high osmolality saline during knee arthroscopic surgery is beneficial to the postoperative repair of damaged cartilage, but there is still a lack of research on the mechanism of anabolic action of cartilage and clinical application of high osmolality. High osmolality promoted chondrocyte anabolism and inhibited catabolism. The osmolality of synovial fluid in patients with OA decreased significantly compared with normal synovial fluid. The decrease of osmolality induced the decline of chondrocyte anabolism, as manifested by downregulation of SOX9, Collagen2a1 and ACAN. By upregulating osmotic pressure, this phenomenon can be reversed. At the same time, high osmolality upregulated Nfat5, Gas1 and SHH signaling which were screened by RNA-seq and the axis of action was formed between Nfat5, Gas1 and SHH signaling. High osmolality decreased Nfat5 mRNA transcription and Nfat5-mediated Gas1 expression, which consequently stimulated SHH signaling. Generally, high osmolality promoted the anabolism of chondrocytes and delayed osteoarthritis by mediating Nfat5-Gas1-SHH axis. A novel high osmotic artificial synovial fluid was injected into the joint cavity of mice, and the sham group, the sham + synovial fluid group, the DMM group, and the DMM+ synovial fluid group were compared. So, our study found that Nfat5-Gas1-SHH axis is a key factor in the association between high osmolality and chondrocyte anabolism and catabolism in the pathology of OA.

Po1_2.25

Do Macrophages have a Dual Role in Cartilage Repair: Priming then Enhancing Chondrogenesis?

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Abstract

Introduction. After joint cartilage injury, poor healing including fibrosis can result. We lack treatments to improve this which can later lead to osteoarthritis. Chronic inflammation, in part attributable to macrophage subtype, contributes to fibrosis and correlates with severity of osteoarthritis. Macrophages have been investigated as a clinical therapy for fibrotic diseases and have been shown to improve outcomes in clinical trials. They exist as a spectrum including pro-(M1) and anti-(M2) inflammatory subtypes. We hypothesised that specific macrophage subtypes could improve chondrocyte function and that this includes pro-inflammatory priming of chondrocytes.

Methods. Human monocytes from human leucocyte reduction cones were steered towards M1 (GM-CSF, TNF α /IFN γ) and M2-type macrophages (M-CSF, IL10) for 7 days in vitro. Phenotype was assessed by flow cytometry: M1 (CD38/CD64/CD80) and M2 (CD206/MERTK/CD163). Influence on chondrogenesis was assessed by applying conditioned media (CM) from M1 or M2 macrophages to isolated human chondrocytes for 24 hours (n=3). Sequential application of M1 CM followed by M2 CM (12 hours each) represented an 'inflammation then repair' model. For protein analysis, media was applied for 48 hours.

Results. Adding M2 CM alone to chondrocytes consistently led to a significant increase in chondrogenic genes including COL2A1 (50-fold increase) and DCN (60-fold increase). When exposed to sequential M1 CM followed by M2 CM, chondrocyte SOX9 expression was dramatically (25-fold) enhanced. Furthermore, increased progenitor-related markers CD105, CD73, CD44 and Notch were observed with M1 CM treatment alone, as well as increased RUNX2, pro-inflammatory cytokine expression levels, and enhanced cell migration. Confocal microscopy of chondrocytes supported these findings by revealing elevated abundance of ACAN, DCN, COL2A1 in M2 CM exposed chondrocytes whereas RUNX2 was increased in M1 CM exposed chondrocytes. Differential gene expression analysis to investigate these findings further and explore underlying gene regulatory pathways is currently in progress using a bulk RNAseq approach.

Conclusions. Sequential application of M1 CM then M2 CM particularly enhanced SOX9 expression, likely leading to enhanced cartilage marker (e.g. COL2A1) expression compared with M2 CM exposure alone at later timepoints. M1 CM led to enhanced migration and progenitor-related markers, possibly indicating de-differentiation of chondrocytes. These findings support our hypothesis that transient acute injury/inflammation influences monocyte subpopulations priming chondrocytes and leading to enhanced responsiveness to subsequent pro-repair signalling.

Po1_2.26

Human osteochondral cylinders as a model to investigate degenerative processes in osteoarthritis

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Abstract

Osteoarthritis (OA) is a common degenerative disease leading to functional limitations and pain in affected joints mainly due to progressive cartilage degradation. Unfortunately, the knowledge obtained from OA animal models cannot always be transferred to humans due to different anatomical and metabolic features. Therefore, the aim of this study was to establish a human tissue culture to investigate early OA-relevant degenerative processes.

Osteochondral cylinders were collected from tibial plateaus of OA patients undergoing knee joint replacement surgery. Cylinders were cultured for up to seven days, in the absence or presence of interleukin-1 β (IL-1 β) and matrix metalloproteinases (MMP)-8 or -13. Safranin-O, DMMB and immunohistochemical (IHC) stainings were used to monitor proteoglycan (PG) loss and the localization of OA-relevant cartilage matrix components (aggrecan, DIPEN aggrecan fragment, collagen II, COMP, fibronectin, thrombospondin-4, matrilin-3 and nidogen-2) over time. Tissue culture supernatants were analyzed by immunoblot to determine release and fragmentation of specific matrix proteins.

Cylinders demonstrated an OA-characteristic progressive PG loss upon treatment with IL-1 β , MMP-8 or MMP-13, while PG levels in controls remained unaltered. IHC stainings showed a general decrease in staining intensity in the superficial and middle zone. All treatments led to a protein redistribution/reexpression in deeper zones. Immunoblots revealed a detectable release of full-length proteins in all samples. However, additional bands were detected in treated samples, indicating a specific fragmentation of matrix components.

This study validates short-term cultures of osteochondral cylinders as a potential preclinical human OA model. The analysis of expression, localization, secretion and fragmentation of selected matrix components demonstrates that alterations accompanied by proteoglycan loss can be detected. This analysis can be extended to any protein/proteoglycan of interest, to better understand their role in OA progression. The bioactivity of specific fragments that were generated upon treatment with IL-1 β , MMP-8 or MMP-13 will be tested in future studies. A comparison of fragmentation patterns with human serum or synovial fluid and the determination of specific cleavage sites might lead to the identification of novel biomarkers. Osteochondral tissue cultures might also be used for high-throughput screening of novel potential disease-modifying OA drugs with the major advantage of using human tissue and at the same time reducing animal experiments.

Po1_2.27

Impact of Triamcinolone Acetonide on Fibroblast-Like Synoviocytes: Insights into Viability, Biomechanical Fingerprints, and Mechanosensitivity

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Abstract

Introduction: Fibroblast-like synoviocytes (FLS), residing within the synovial membrane, are pivotal in maintaining joint homeostasis through the production of synovial fluid, immune regulation, and tissue repair.² In osteoarthritis (OA), a joint-degenerative disease characterized by elevated pro-inflammatory mediators, FLS play a central role in their secretion.² The progression of (OA) is marked by dysregulation of mechanosensitivity and mechanotransduction in fibroblast-like synoviocytes (FLS).¹ Corticosteroids, such as triamcinolone acetonide (TA), a widely used clinical agent, are employed to alleviate OA-associated pain by targeting inflammation.³ This study investigates the effects of TA on FLS, focusing on cellular viability, biomechanics, mechanosensitive ion channels, and cytoskeletal dynamics.

Method: Synoviocytes were isolated from the synovial membrane of OA patients (n=3) undergoing total knee replacement and incubated with varying concentrations of triamcinolone (TA). Cell viability and morphology were assessed using fluorescence imaging, flow cytometry (FACS), and a viability assay. Atomic force microscopy (AFM) was employed to measure cell elasticity. Additionally, mechanical compression (500nN) via AFM was applied to cells pre-labeled with Fluo-4 (a calcium indicator) to investigate mechanically induced calcium dynamics. Lastly, the expression of cytoskeletal components (F-actin) and mechanosensitive ion channels (Piezo1, Piezo2, TRPV4) was analyzed to explore the interactions between these channels and the cytoskeleton in regulating calcium dynamics.

Result: Treatment with TA induced notable morphological changes in FLS cells, with these alterations diminishing at lower TA concentrations. Interestingly, cell viability improved as TA concentrations decreased. Elasticity measurements showed that lower TA concentrations significantly increased cellular stiffness ($p=0.0219$), while higher TA concentrations had minimal effect on cell elasticity ($p=0.5391$). Additionally, mechanical-induced calcium influx was notably altered by TA treatment, with these changes further supported by corresponding shifts in gene expression.

Conclusion: Treatment with TA significantly impacts FLS, altering their cell morphology, viability, and elasticity while also disrupting calcium homeostasis under mechanical stimulation. These changes are further validated by corresponding shifts in the expression

of structural and mechanosensitive markers, highlighting the broad cellular implications of corticosteroid treatment.

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Po1_2.29

Clinical outcomes following medial meniscus posterior root repairs: A minimum of 5-year follow-up study

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Abstract

Purpose: This study assessed the clinical outcomes after Fast-Fix dependent modified Mason-Allen suture (F-MMA) and two simple stitches (TSS) on mid-term postoperative outcomes following medial meniscus posterior root tear (MMPRT) repairs.

Methods: Forty-three patients who underwent transtibial pullout repair for MMPRT between November 2016 and September 2018 were initially enrolled. Patients with a femorotibial angle (FTA) <180°, a Kellgren-Lawrence grade of 0–2, and modified Outerbridge grade I or II cartilage lesions were included. Lysholm score, Tegner activity, International Knee Documentation Committee score, pain visual analogue scale, and Knee injury and Osteoarthritis Outcome scores were assessed as clinical outcomes. Conversion surgery to knee arthroplasty was set as the end point. Surgeries other than second-look arthroscopy and plate or screw removal were also recorded.

Results: At 5 years of follow-up, there were 28 cases, and at 6 years, 15 cases, with a mean follow-up of 5.3 ± 0.5 years. All evaluated 5-year postoperative clinical outcomes were significantly improved compared with the preoperative outcomes ($p < 0.001$). Both F-MMA and TSS significantly improved all clinical scores at 5 years postoperatively in patients with MMPRT, whereas the F-MMA and TSS groups showed no significant differences in pre- and postoperative clinical scores. None of the patients required ipsilateral knee arthroplasty during the follow-up, and the survival rate after pullout repair was 100%, whereas the rates of subsequent knee-related surgical treatment were 11.6% in the pullout-repaired knees, including an additional all-inside suture repair, partial meniscectomy, and arthroscopic debridement for arthrofibrosis with a limited range of motion.

Conclusion: Both F-MMA and TSS pullout repair yielded satisfactory clinical outcomes in patients with MMPRT, with a mean follow-up of 5.3 years, and no conversions knee arthroplasty were required. Further follow-up is warranted to assess the long-term survival rates.

Po1_2.30

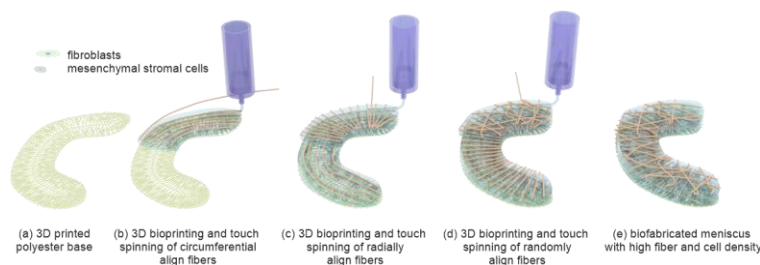
Fiber-Reinforced Self-Healing Gels for Meniscus Tissue Engineering

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Abstract

The meniscus tissue plays a crucial role in force transmission, shock absorption, joint lubrication, and joint stability. The primary challenge in the biofabrication of the meniscus lies in its highly complex structure, which consists of uniquely distributed and aligned fibers, glycosaminoglycans, and high-water content. This distinct structure endows the meniscus with exceptional mechanical properties, unmatched by any other tissue in the body, enabling it to withstand high loads during activities such as jumping, climbing, and running. Tissue engineering approaches have often relied on highly brittle crosslinked hydrogels and fibers that fail to replicate the size, arrangement, and mechanical behavior of natural collagen. In this study, we present a novel approach that combines self-healing gels to mimic the aqueous portion of the meniscus and polyhydroxybutyrate (PHB)-based fibers arranged in a manner that closely resembles natural collagen tissue. The use of self-healing gels reduces the risk of creep formation due to their superior mechanical properties. The dynamic bonds in self-healing gels allow for rapid structural reconstruction (within 10 minutes) and result in a two-fold reduction in the loss factor compared to conventional hydrogels. Furthermore, with the implementation of advanced 4D-Fibroprinting technology (developed by bioature GmbH), we can automatically fabricate structures that closely mimic native tissues, incorporating a high density of fibers aligned to replicate the natural architecture of the meniscus. The resulting structures exhibit enhanced mechanical strength and a closer resemblance to natural meniscus tissue, offering significant potential for improved tissue engineering solutions.



Po1_2.31

Nano mineral crystals perturbation from osteochondral interface initiates osteoarthritis

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Abstract

As a common degenerative joint disease, osteoarthritis (OA) has been thoroughly studied for its pathogenesis, including cell phenotype and histological morphology changes. Yet, the nano-micro scale spatiotemporal pathological progression, especially its original site and actuating events during OA progression remain largely unknown. Here, we firstly demonstrate that the OA progression initiates from osteochondral interface by nano mineral crystals perturbation. Nanocrystals deposition increases mechanical stiffness of extracellular matrix (ECM), which upregulate fibronectin and vitronectin expression from hypertrophic chondrocytes. Hyper-mineralized vesicles released by these cells facilitates the ECM bottom-up mineralization and top-down degeneration. Furthermore, mechanical overload removal can block the ECM mineralization and degradation, and effectively reverse the OA pathology. The present work advances our comprehension of the underlying mechanisms of OA progression and offers a foundation for potential OA therapeutic strategies.

Po1_2.32

Coaxial 3D Printing: synergistic approach of natural and synthetic biomaterials for knee meniscus replacement

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Abstract

Knee meniscus lesions are a common injury that can significantly impact an individual's quality of life [1]. Current treatments for total meniscus replacement are unavailable, and the partial replacement alternatives provide only short-term relief, increasing the risk of knee arthritis in the long run. However, advancements in knee meniscus implants and 3D printing technology have the potential to revolutionize the treatment of knee meniscus lesions [2]. These novel implants should be capable to withstand physiological forces while promoting efficient tissue integration.

This project uses a coaxial printing approach which allows for the simultaneous extrusion of two materials through a single nozzle. The materials are extruded in a coaxial manner, meaning that they are aligned along the same axis and flow out of the nozzle together, producing a 3D printed strand composed by a core and a shell (Figure 1). This synergistic approach allows for the combination of natural and synthetic biomaterials in a fast-setting strategy, targeting to develop an ink for 3D printing with both good mechanical performance and biocompatibility.

In this work, we focus on using a synthetic-based material for the core, aiming to withstand physiological forces and be mechanically suitable for meniscus application, and natural based-biomaterial for the shell to promote cell attachment and further tissue integration. Polycaprolactone and polyethylene glycol diacrylate have been tested as core materials and silk fibroin (SF) as shell. This innovative coaxial 3D printing was developed, and the produced scaffolds were characterized in terms of physico-chemical and biological properties. The architecture of the scaffolds was observed using a scanning electron microscope (SEM). Tensile and compressive uniaxial tests were performed as well as *in vitro* cytotoxicity assays.

Through this innovative technique, an innovative coaxial ink with suitable mechanical properties and biological performance was developed for meniscus tissue engineering.

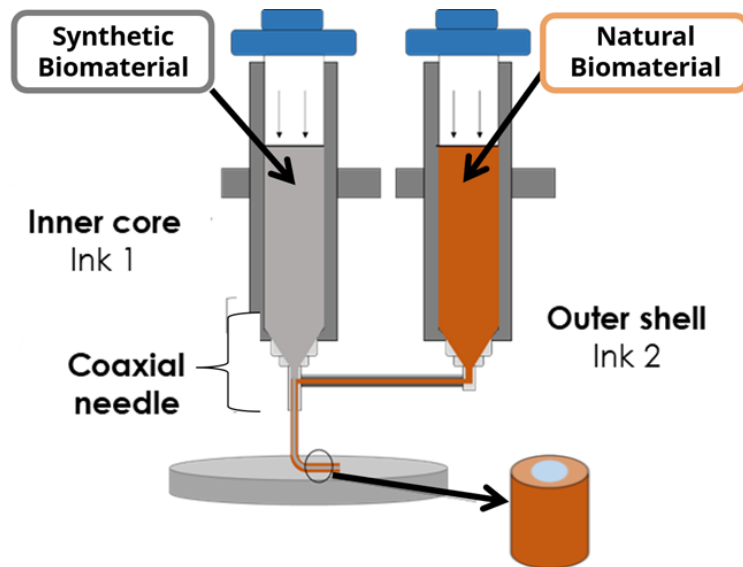


Figure 1 – Scheme of coaxial setup.

Acknowledgements: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through the Doctoral Research Grant 2024.00742.BD attributed to FAPR. The authors would like also to acknowledge the support of Project No. IBEROS+ (0072_IBEROS_MAIS_1_E. JBC is grateful to FCT for the individual Junior Researcher contract (org.doi:10.54499/2022.02781.CEECIND/CP1745/CT0001).

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Po1_2.33

Impact of glucose and oxygen on osteochondral-synovium co-culture

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Abstract

Introduction

There are several challenges in using *ex vivo* and *in vitro* culture models in cartilage research. Monocultures fail to replicate the complex interactions between cartilage, synovium, and subchondral bone. Additionally, glucose and oxygen levels vary significantly across studies, with no standardized protocols. This study aims to develop an *ex vivo* co-culture system with osteochondral and synovium tissues and investigate the impact of glucose levels in the establishment of an *ex vivo* joint model under different oxygen conditions.

Methods

Bovine osteochondral explants (8 mm in diameter, 6 mm in height) and synovium (8 mm diameter discs) were collected from the stifle joint. *Ex vivo* co-cultures were conducted in two different culture media: DMEM high glucose (HG-4500 mg/ml), and DMEM low glucose (LG-1000 mg/ml) under hyperoxia (21% O₂) or physioxia (5% O₂) conditions. During 7 days of culture, release of glycosaminoglycan (GAG) and nitric oxide (NO) were measured using Dimethylmethylene Blue and Griess assay, respectively. Expression of mRNA levels were measured by RT-qPCR. Cell viability in cartilage and synovium was evaluated using Live/Dead staining. Experiments were performed with three biological replicates.

Results

Live/dead staining results indicated that LG media lead to increased cell death in the cartilage deep zone if the cartilage thickness was more than 1 mm. GAG release was independent of both glucose and oxygen levels, with no significant differences observed between the groups. Moreover, NO release increased in tissues cultured in HG media only in physioxia condition. PCR results showed that in cartilage, the expression of ACAN, ADAMTS4, and IL6 mRNA was higher in DMEM HG compared with DMEM LG only in hyperoxic condition. In bone, glucose levels had no detectable effect on ALPL and VEGF expression. However, 5% O₂ downregulated expression of ALPL and upregulated expression of VEGF compared to 21% O₂. In both cartilage and bone tissues, LG media increased the expression of GLUT3 compared to HG medium in both hyperoxia and physioxia.

Conclusions

This study revealed that glucose levels affect cell viability in *ex vivo* cartilage cultures. Cartilage and bone tissues showed more stable gene expression under physioxenic conditions, likely due to their closer resemblance to *in vivo* environments. For cartilage thicker than 1mm, HG medium ensures optimal cell viability. For thinner cartilage, both LG and HG are suitable under physioxenic conditions for *ex vivo* joint tissue cultures. If physioxenia is unavailable, LG medium is recommended to maintain tissue homeostasis.

Po1_2.34

α -MSH modulates senescent and metabolic state of human osteoarthritic and non-osteoarthritic chondrocytes

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Abstract

Introduction: Osteoarthritis (OA) is a degenerative joint disease, where age is a major risk factor. OA causes structural damage in all joint tissues, with inflammatory mediators playing a critical role. The α -melanocyte-stimulating hormone (α -MSH) is a melanocortin (MC) peptide, which mediates its functions via MC receptors (MCR). Beyond pigment induction, α -MSH has several anti-inflammatory and chondro-protective effects dampening inflammatory and presumably age-related processes. Abrogated MC1R signalling leads to a more severe OA-pathology in a murine surgical OA model. The underlying molecular mechanisms as signalling pathways, intracellular effectors and influence of ageing are mostly unknown yet. Therefore, the aim of this study was to analyse the role of α -MSH in senescent human OA chondrocytes.

Methods: Chondrocytes were isolated from human OA and non-OA (cadaver) knee cartilage explants. Chondrocytes, cultivated as 2D monolayers, were incubated with α -MSH and treated with doxorubicin (doxo) to induce a senescent phenotype. Induction of senescence was verified by senescence-associated β -galactosidase (SA- β -Gal) activity, apoptosis and cytotoxicity levels, and qPCR. Metabolic activity was analysed by mitochondrial dehydrogenases activity, ATP activity, and oxidative stress level. The expression and secretion of cytokines, matrix metalloproteinases (MMP) and complement markers were analysed in cell lysates and supernatants by qPCR and Luminex Multiplex-ELISA. All tested human chondrocytes were genotyped for MC1R loss-of-function polymorphisms (SNPs). MC1R expression and polymerization in SNP and non-SNP samples were examined by qPCR and Western Blot.

Results: The expression of SA- β -Gal was increased in both, OA-and non-OA chondrocytes treated with doxo, while α -MSH slightly reduced senescence, whereas we did not observe changes in apoptosis or cytotoxicity. We detected an increase in CDK-inhibitors CDKN2A and CDKN1A, increased IL6, MMP13, complement components C3 and CFB mRNA expression in doxo-treated chondrocytes, whereas chondrogenic marker expression of SOX9, ACAN, COL2A1 and RUNX2 was decreased. Senescent chondrocytes incubated with α -MSH showed decreased protein secretion levels

of MMP-13, C3b and anaphylatoxin C3a compared to doxo-treated cells. Oxidative stress levels were increased in senescent chondrocytes, whereas α -MSH normalized this effect to that of untreated cells. Senescent chondrocytes showed increased metabolic activity, with α -MSH potentiating these effects in both OA- and non-OA samples. We detected different MC1R SNPs in 70% of the genotyped OA- and in 40% of the non-OA chondrocytes.

Discussion: Our in vitro data indicated anti-ageing and anabolic effects of α -MSH on senescent human OA- and non-OA-chondrocytes. The impact of α -MSH signalling on chondrocytes with MC1R SNPs is of interest for understanding α -MSH molecular effects.

Po1_2.35

An ex vivo calcified tissue explant model to study the response of cells in the cartilage-bone interface to osteoarthritic factors

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Abstract

Calcified cartilage connects the articular cartilage and subchondral bone is hypothesized to play a role in cartilage homeostasis and osteoarthritis (OA). The low cell density of 75-150 cells per mm² [PMID:35032407] and its mineralized thin tissue layer of 75 – 250 µm [PMID:7410471] are the main challenges in processing and analysing this tissue. The aim of this project was to set-up a calcified tissue explant culture model to study the response of the cells residing in the calcified tissue to factors associated with OA.

Patient-derived explants of articular cartilage with a thin layer of subchondral bone were harvested (n=4 donor) from patients with OA undergoing knee replacement. The calcified tissue interface was isolated by enzymatic digestion with collagenase B to remove bone lining cells and to remove the non-calcified cartilage. Micro-computed tomography (CT) imaging and histology (Safranin-Orange staining) were performed to characterize the calcified tissue explants. Full thickness non-calcified cartilage explants served as control samples. The explants were cultured in basic media (DMEM-LG, antibiotics, 1% ITS⁺) and stimulated with either an inflammatory cytokine cocktail (TNF-α, IL-1β, INF-γ, 1ng/ml) or TGF-β1 (10ng/ml) for three days. qPCR data of stimulated calcified and non-calcified explants was compared to data from donor matched explants cultured in control media without stimulation.

Using the enzymatic digestion protocol, we succeeded in obtaining explants of calcified tissues that comprised the zone of calcified cartilage with a thin layer of the adjacent subchondral bone based on CT scans and histology. Cells residing in the calcified tissue explants distinguished from chondrocytes in the non-calcified cartilage explants by a higher relative expression in *COL10A1*, *COL1A1*, *SPP1* and *RUNX2* ($p \leq 0.0405$). Cartilage associated genes *COL2A1* and *ACAN* were less expressed in the calcified explants than in the non-calcified cartilage explants ($p < 0.0139$). The stimulation with cytokines evoked an increase in expression of *MMP13* ($p < 0.0431$) and *IL6* ($p < 0.0294$) in both calcified and non-calcified explants compared to the donor matched control samples. The stimulation with TGF-β significantly increased *COL10A1* ($p < 0.0340$) in calcified explants compared to the respective explants in control media without stimulation. Non-calcified explants responded to cytokine treatment with an increase in *MMP13* ($p = 0.0431$) and a decrease in *COL2A1* and *ACAN* ($p < 0.0263$).

This novel calcified tissue explant model demonstrated that this layer is sensitive to factors associated with OA disease progression. This model holds promise to investigate molecular mechanisms and pathologic changes at the cartilage-bone interface to decipher its role in OA disease progression.

Po1_2.36

Differentially Expressed Genes and Pathways between Degenerated and Healthier Human Articular Cartilage

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Abstract

Introduction: Osteoarthritis (OA) is characterized by progressive degeneration of articular cartilage, leading to joint dysfunction and pain¹. Identifying molecular differences between healthy and degenerated cartilage could improve our understanding of disease mechanisms and reveal novel therapeutic targets. Here, we present bulk RNA sequencing data comparing gene expression in healthy and degenerated cartilage regions from patients undergoing total knee replacement surgery.

Methods: Articular cartilage samples were collected from six patients and dissected into healthy and degenerated regions. Samples were enzymatically digested using pronase and collagenase II, and isolated cells were shock frozen into pellets. RNA was extracted and subjected to bulk RNA sequencing using Illumina NovaSeq X Plus (paired-end 150 bp). Reads were filtered and aligned using Fastp, and differentially expressed (DE) genes were identified using DESeq2, applying thresholds of $|\log_2\text{FoldChange}| \geq 1.5$ and adjusted p-value < 0.05 . Pathway enrichment analyses were conducted using PROGENy, Gene Ontology (GO), KEGG, and WikiPathways.

Results: Significant batch-to-batch variation and inter-patient variability were observed. DGE analysis identified a subset of genes with significant expression differences between healthy and degenerated cartilage. Upregulated genes in degenerated cartilage were enriched in pathways related to extracellular matrix (ECM) degradation (e.g., ADAMTS family genes), inflammation (e.g., RIPK4), and oxidative stress (e.g., DUOX1). Downregulated genes included those associated with ECM maintenance (e.g., COL10A1, UCMA) and anti-inflammatory responses (e.g., DKK1).

IL1RL1, however, which is also associated with inflammation, was upregulated in all healthy samples. This might reflect tissue deterioration within the healthy tissue by the presence of pro-inflammatory cytokines.

Wnt, TNF- α , p53, MAPK, and TGF- β signaling pathways were enriched in degenerated cartilage. Moreover, the Notch signaling and ECM integrity-related pathways were suppressed. These findings align with the known pathophysiology of OA, highlighting the interplay between inflammation, ECM remodeling and impaired repair mechanisms.

Conclusion: Our transcriptomic analysis identified key molecular differences between healthy and degenerated cartilage, despite the batch and inter-patient variability, supporting the notion of inflammation driving cartilage degeneration. The data suggested the involvement of inflammatory signaling and ECM degradation during OA progression. This study provides valuable insights into disease mechanisms and establishes a baseline for distinguishing healthy and degenerated cartilage, facilitating the development of targeted therapeutic approaches and tissue engineering strategies.

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<https://doi.org/10.1097/01.blo.0000143938.30681.9d>

Po1_2.37

MODELLING MENISCAL DEGENERATION: A 3D CULTURE PLATFORM FOR STUDYING INFLAMMATORY AND MATRIX RESPONSES

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Abstract

Introduction: Pro-inflammatory cytokines such as interleukin (IL)-1 β and tumour necrosis factor (TNF)- α are key mediators of inflammatory following meniscal injury (McNulty et al., 2013; Ogura et al., 2016; McNulty et al., 2007). Currently, few models replicate ex vivo the three-dimensional degenerative environment to study meniscus cells response to tissue engineering (Perea et al., 2020). Hence, this study established a degenerative human meniscus culture model to simulate a native pro-inflammatory/degenerative environment.

Methods: Based on the Kellgren and Lawrence (KL) classification, mildly (n=12, KL 1-2) and severely (n=12, KL 3-4) degenerated human menisci were analysed for DNA, glycosaminoglycans (GAG), total collagen, aggrecan, collagen type I (COL1), COL2, IL-6 and matrix metalloproteinase (MMP)-3 using biochemical assays or immunohistochemistry. Mildly degenerated menisci (n=7, KL 1-2) were cultured for 21 days under basal conditions (control) or treated with 10 ng/mL recombinant human i) IL-1 β , ii) TNF- α or iii) IL-1 β +TNF- α . Cell viability, GAG, collagen, aggrecan, COL1, COL2, IL-6, and MMP-3 levels were evaluated. Kruskal-Wallis tests determined statistical significance (p<0.05).

Results: Severely degenerated menisci showed significantly higher aggrecan, IL-6 and MMP-3 levels than mildly degenerated samples (p<0.05), with no differences in COL1 or COL2. After 21 days of basal culture, mildly degenerated tissues maintained cell viability and DNA content but exhibited reduced GAG and collagen levels, resembling severely degenerated menisci. IL-6 and MMP-3 production remained low in basal conditions, similar to native mildly degenerated samples. Treatment with IL-1 β or TNF- α did not affect cell viability, GAG (Fig. 1A), or total collagen content (Fig. 1B). Notably, IL-1 β stimulation did not alter aggrecan content (Fig. 1C) but induced a shift from COL2 to COL1 production (p<0.05, Fig. 1D,E) and significantly increased IL-6 and MMP-3 (p<0.05, Fig. 1F,G), reflecting a degenerative matrix profile.

Conclusion: Under basal conditions, meniscus cultures retained viability but lost GAG and collagen, resembling severely degenerated tissues. IL-1 β treatment simulated a pro-inflammatory environment by increasing IL-6 and MMP-3 production and shifting collagen production from COL2 to COL1. Similar effects have been shown with IL-1 treatment of porcine meniscal explants (McNulty et al., 2007). This model successfully mimics the

degenerative features of native menisci, offering a platform to study tissue engineering approaches for meniscal repair. Funding: ON Foundation (21-340).

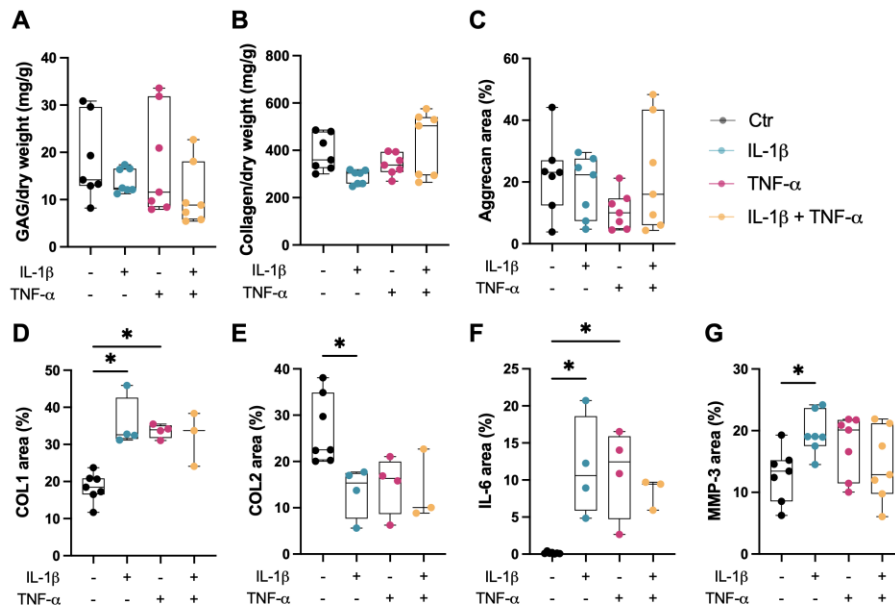


Figure 1. Human meniscus tissues were analyzed at day 21 of culture under control conditions or treated with 10 ng/mL IL-1 β , TNF- α , or IL-1 β +TNF- α . **A)** GAG and **B)** collagen normalized to dry weight (mg/g) in meniscal tissues. **C)** Aggrecan, **D)** COL1, **E)** COL2, **F)** IL-6, and **G)** MMP-3 area (%). n=3-7, Kruskal-Wallis test.

Po1_2.38

DYNAMIC LOADING ENHANCES COLLAGEN-RICH MATRIX FORMATION IN PET SCAFFOLDS FOR MENISCAL REPLACEMENT

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Abstract

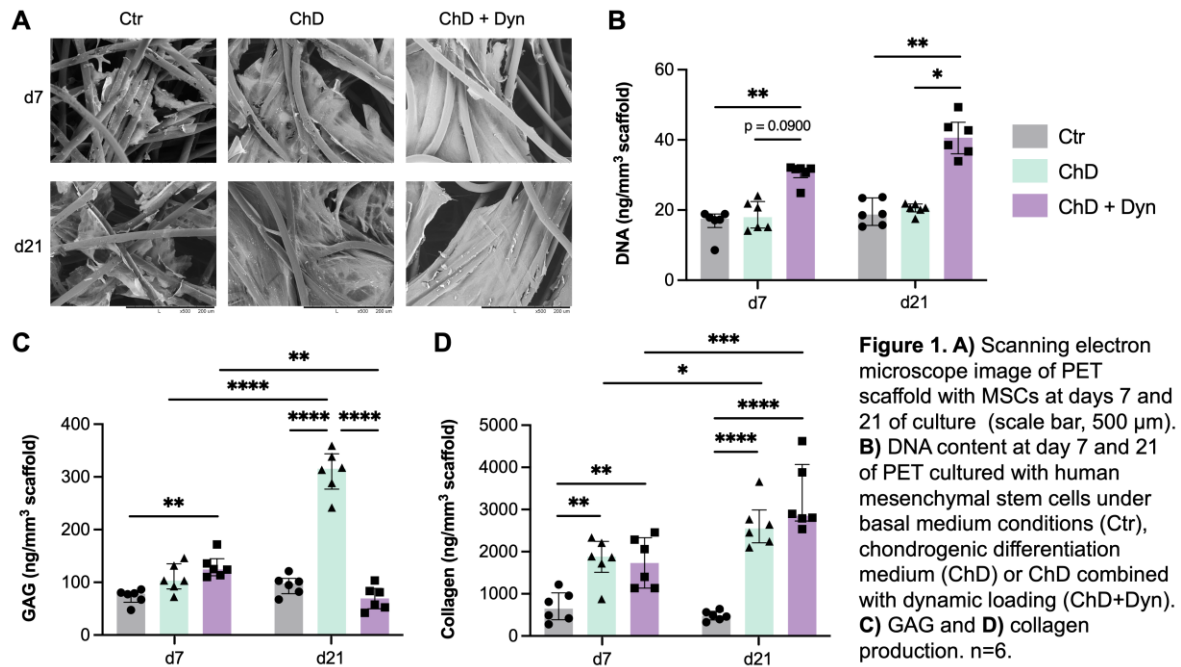
Introduction: The meniscus plays a critical role in protecting articular cartilage in the knee but is prone to damage from sports injuries and age-related degeneration. Tissue engineering offers a promising solution for meniscal replacement, yet identifying an ideal biomaterial remains challenging. Consequently, this study aimed to characterise a novel polyethylene terephthalate (PET) scaffold for partial meniscal replacement.

Methods: Non-woven PET scaffolds (400-420 g/mm²) were analysed for porosity and fibre orientation by μ CT and SEM. Human mesenchymal stem cells (MSCs) were seeded onto scaffolds and cultured for 7 or 21 days under: i) basal medium (Ctr), ii) chondrogenic medium (ChD) or iii) ChD with dynamic loading (ChD+Dyn). Physiologic loading (12% strain, 1 Hz, 1h/day, 5 days/week) was applied using a custom-made device. Metabolic activity was assessed with resazurin assay. Glucose and lactate levels were quantified by biochemical assays in culture supernatants. DNA, glycosaminoglycan (GAG), and collagen were measured in digested constructs. Biomechanical properties, including equilibrium modulus (E_{eq} , kPa) and permeability (k , $10^{-15} \text{m}^4 \text{N}^{-1} \text{s}^{-1}$), were determined using multistep-confined compression testing. Two-way ANOVA was used for statistical analysis ($p < 0.05$).

Results: PET scaffolds demonstrated $85.2 \pm 0.5\%$ porosity, interconnecting pores, and uniform cell distribution. MSCs remained metabolically active under all conditions tested and matrix production increased over time in culture (Fig. 1A). ChD+Dyn significantly increased DNA content on day 21 ($p < 0.05$, Fig. 1B) but decreased glucose consumption and lactate production at both time points ($p < 0.0001$) compared to Ctr and ChD. GAG content was significantly lower in ChD+Dyn than in ChD on day 21 ($p < 0.0001$, Fig. 1C), while collagen production was similar (Fig. 1D). Notably, GAG content decreased ($p < 0.01$) and collagen increased ($p < 0.001$) over time in ChD+Dyn cultures. No significant differences in E_{eq} or k were observed across groups.

Conclusions: MSCs remained viable over 21 days, and dynamic loading in chondrogenic conditions promoted collagen-rich, meniscus-like matrix production, albeit with lower GAG content. Despite increased collagen, biomechanical properties did not differ between groups, suggesting the produced matrix was insufficient to impact scaffold stiffness or permeability within 21 days. This study demonstrates the potential of PET scaffolds for

meniscal replacement and highlights the benefits of dynamic loading for improving construct functionalisation. Funding: DFG (SE 3135/3-1).



Po1_2.39

Injectable Bilayer Elastin-Like Recombinamer Bioactive Hydrogels with Differential Layer Degradation Rates for Osteochondral Regeneration in an In Vivo Rabbit Model

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Abstract

The repair of osteochondral defects poses a considerable challenge due to the unique structural and functional characteristics of cartilage and subchondral bone. Current approaches often employ multiphasic scaffolds designed to replicate the intricate architecture of the osteochondral unit. In this work, we introduce an injectable hydrogel system specifically engineered with distinct inner and outer layers, each characterized by unique degradation rates and cellular compositions, to support the regeneration of critical-sized osteochondral defects in a rabbit model over an extended period.

This hydrogel platform is constructed from elastin-like recombinamers (ELRs), which provide mechanical stability and foster cell attachment and proliferation. The design incorporates functional sequences, including RGD motifs to promote cell adhesion and protease-sensitive sites with different degradation profiles—slow-cleaving DRIR sequences and fast-cleaving GTAR sequences. Delivered in liquid form, the hydrogels solidify directly at the defect site, seamlessly adapting to the shape of the lesion.

To enhance the regenerative process, various cell types, such as mesenchymal stem cells (MSCs) and chondrocytes, were integrated into the hydrogel layers. After six months of implantation, the outcomes were evaluated using glycosaminoglycan quantification and histological techniques, both of which confirmed the production of cartilage-like tissue. Statistical analysis using the modified O'Driscoll score demonstrated significant improvements ($p < 0.05$) in the homogeneous hydrogels (with either fully fast-degrading or slow-degrading layers) compared to untreated controls and hydrogels containing only chondrocytes. While other combinations did not show statistical differences based on the O'Driscoll score, histological assessments revealed promising cartilage-like tissue development, even in hydrogels without embedded cells.

This strategy highlights the potential of an in situ crosslinking hydrogel system for osteochondral defect repair. The approach achieves notable cartilage-like tissue regeneration (Figure 1), even in the absence of cells, while the inclusion of MSCs and

chondrocytes further improves outcomes. This versatile system offers a promising pathway for treating complex osteochondral lesions effectively.

Table 1. Hydrogel combinations used in this study, alternating two different degradation rates (Fast (F) and Slow (S)) and two cell types (mesenchymal stem cells (m) and chondrocytes (c)).

	INTERNAL ZONE		EXTERNAL ZONE	
	HYDROGEL	CELLS	HYDROGEL	CELLS
F_mF_c	<u>Fast</u>	MSC	<u>Fast</u>	<u>Chondrocytes</u>
S_mS_c	<u>Slow</u>	MSC	<u>Slow</u>	<u>Chondrocytes</u>
F_mS_c	<u>Fast</u>	MSC	<u>Slow</u>	<u>Chondrocytes</u>
S_mF_c	<u>Slow</u>	MSC	<u>Fast</u>	<u>Chondrocytes</u>
S_mF_m	<u>Slow</u>	MSC	<u>Fast</u>	MSC
S_cF_c	<u>Slow</u>	<u>Chondrocytes</u>	<u>Fast</u>	<u>Chondrocytes</u>
S_mS_m	<u>Slow</u>	----	<u>Slow</u>	----
Control	----	----	----	----

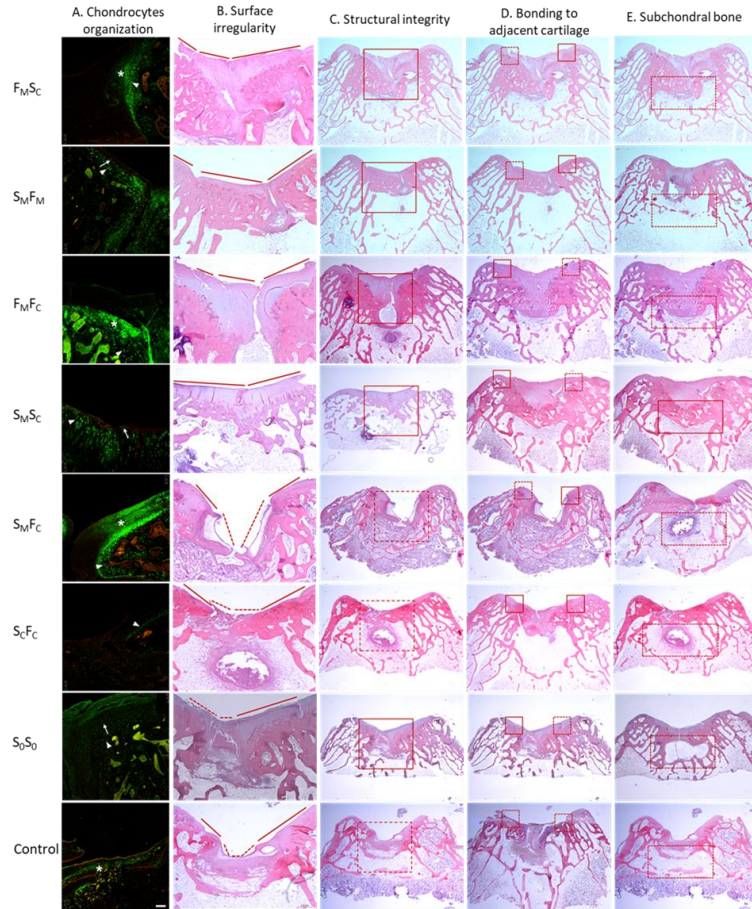


Figure 1. (A) Images showing chondrocytes organization on each experimental series. Head arrows rounded cells with CSPG in lacunae. Arrows pointing elongated cells, aligned parallel to the surface with CSPG lacunae very small or non-existent. Asteristics pointing CSPG in extracellular matrix. (B) Images showing Surface regularity on each experimental series. Discontinuous red lines pointing fissures at the middle of the injury. Pointed red lines pointing no cartilage, or severe disruption at the surface (C) Images showing structural integrity on each experimental series. Squares with continuous red line indicate slight disruption. Squares with discontinuous red line indicate severe disruption. (D) Images showing new cartilage bounding to adjacent cartilage on each pointing experimental series. Red squares with continuous red line indicate bounded cartilage. Red squares with discontinuous red line indicate unbounded cartilage. (E) Images showing reconstruction of subchondral bone on each experimental series. Squares with continuous red line indicate subchondral bone totally reconstructed. Squares with discontinuous red line indicate subchondral bone partially reconstructed. Scale bar: 100µm

Po1_2.41

Uncovering the Impact of Irrigation Fluid Acidity on Articular Cartilage in an ex Vivo Injury Model

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Abstract

Introduction. Arthroscopy is a common procedure in orthopedics and sports medicine, particularly for addressing knee joint symptoms. Various irrigation solutions are used during arthroscopy, although their impact on cartilage homeostasis is not well understood. While previous studies have explored the effect of irrigation solution osmolality, research on the impact of pH levels remains limited. Typical irrigation solutions (saline pH 5.5, Ringer's pH 6.5) have a significantly lower pH than that of a normal joint's synovial fluid (pH 7.3). This study aimed to investigate the effect of different pH levels on cartilage in an injury model.

Methods. Osteochondral explants 8 mm in diameter were harvested from bovine knee joints and full-thickness cartilage was damaged with a blade. Samples were pre-cultured for 2 days in DMEM supplemented with 10% FBS ± IL-1 β to simulate inflammation. Samples were then exposed to Ringer's solutions with pH values of 5.5, 6.5, or 7.3 for 3 hours, followed by a 3-day incubation in chondropermissive medium (DMEM high glucose, insulin-transferrin-selenium, non-essential amino acids, ascorbate-2phosphate) ± IL-1 β . Cell viability, gene expression, GAG, and NO release were assessed via live/dead staining, RT-qPCR, DMMB, and Griess assays, respectively.

Results. Live/dead staining showed that the majority of cartilage cells remained alive throughout the 3 days of culture. Dead cells were observed across the damage site, confirming physical injury through the full cartilage thickness. Treatment with IL-1 β increased the release of both GAG and NO after 3 days of culture. No significant differences were observed in either GAG or NO release among groups treated with different pH levels. Furthermore, gene expression analysis indicated no significant changes in the expression of COL2, PRG4, ACAN, MMP3, and IL-6 among samples treated with different pH levels, in the presence or absence of IL-1 β .

Conclusion. Since no impact on cartilage GAG and NO release or cartilage-associated gene expression was observed, the different pH levels of irrigation fluids are unlikely to affect arthroscopy outcomes. This conclusion is supported by both short-term analyses immediately after irrigation and longer-term analysis after 3 days of culture. However, due to the limited number of replicates, further experiments are necessary to confirm these findings.

Po1_2.42

Multi-omics reveals cellular interactions in knee osteoarthritis

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Abstract

Purpose

This study aims to explore cellular interactions among knee osteoarthritis tissues using bioinformatics data analysis, with the aim of identifying key interacting molecules that drive the pathological progression of the disease. The findings may offer new insights and potential therapeutic targets for osteoarthritis treatment.

Methods

In this study, we screened the currently available single-cell/single-nucleus RNA sequencing data to ensure that the groupings were appropriate. Cellular interactions between tissues in different states were inferred using the CellChat package. Ligands with significantly altered interaction probabilities in the OA state were identified as key molecules involved in cellular interactions in OA. These ligands were then integrated with synovial fluid proteomics data and osteoarthritis molecular classification markers to identify potential ligands involved in the pathological progression of OA. The changes in expression of these ligands and their corresponding receptors were validated using immunofluorescence.

Results

In this study, A number of key ligand molecules (e.g. FN1, TNC, TNXB, POSTN, IGBP3, SPP1, MIF, etc.) have been identified as being important in the pathological process of OA. This study also further clarified and validated marker proteins and their role in the molecular classification of knee osteoarthritis.

With the advancement of various omics techniques, the pathophysiological processes of many diseases have been increasingly elucidated. In contrast to other diseases, OA is a multi-tissue, whole-joint disease, in which all tissues interact and contribute to the pathological progression. However, few studies have focused on the role of extracellular protein molecules, and even fewer have explored protein molecule interactions across tissues. Therefore, it is essential to investigate the roles of different molecules in cellular interactions among various tissues. This will provide valuable insights into the pathological progression of OA and offer new strategies for its treatment and prevention.

The receptor-ligand database is limited, which has resulted in uncertainty regarding the receptors of numerous molecules. It is essential that future research focuses on identifying the corresponding receptors of these ligand molecules and elucidating their roles in the progression of OA.

Conclusion

The present study involved the screening and validation of several ligand proteins that may play crucial roles in the pathological progression of OA and the identification of marker genes for the molecular classification of knee OA. These findings provide insights into the pathophysiological processes among the tissues of knee OA and offer new prospects for research into the diagnosis and treatment of OA patients with different grades and classifications.

Po1_2.44

Mechanism exploration of Notoginsenoside R1 delaying osteoarthritis

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Abstract

The purpose of the study is to explore whether Notoginsenoside R1 has a protective effect on cartilage and its mechanism of action. This study first confirmed the protective effect of Notoginsenoside R1 on protein level through in vitro experiments, which can promote cartilage synthesis and improve the catabolic disorder of chondrocytes. By using the DMM-induced OA model, it was also demonstrated in vitro that Notoginsenoside R1 reduces osteophyte formation, promotes cartilage recovery, and reduces the pain level of mice during exercise. Finally, we explored downstream targets and pathways through RNA-seq. This study shows that the small molecule compound Notoginsenoside R1 extracted from traditional Chinese medicine can promote cartilage formation and alleviate catabolism disorders, which may provide a new direction for the clinical treatment of OA.

Po1_2.47

Hnrnpk Protects Against Osteoarthritis through Targeting WWC1 mRNA and Inhibiting Hippo Signaling Pathway

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Abstract

Osteoarthritis (OA) is an age-related or post-traumatic degenerative whole joint disease characterized by the rupture of articular cartilage homeostasis, the regulatory mechanisms of which remain elusive. This study identifies the essential role of Heterogeneous nuclear ribonucleoprotein K (hnRNPK) in maintaining articular cartilage homeostasis. Hnrnpk expression is markedly downregulated in human and mice OA cartilage. The deletion of Hnrnpk effectively accelerates the development of post-traumatic and age-dependent OA in mice. Mechanistically, the KH1 and KH2 domain of Hnrnpk bind and degrade the mRNA of *WWC1*. Hnrnpk deletion increases *WWC1* expression, which in turn leads to activation of Hippo signaling and ultimately aggravates OA. In particular, intra-articular injection of LPA and adeno-association virus serotype 5 expressing *WWC1* RNAi ameliorates cartilage degeneration induced by Hnrnpk deletion, and intra-articular injection of adeno-association virus serotype 5 expressing Hnrnpk protects against OA. Collectively, this study firstly reveals the critical roles of Hnrnpk in inhibiting OA development through *WWC1*-dependent downregulation of Hippo signaling in chondrocytes and defines a potential target for the prevention and treatment of OA.

Po1_2.49

Magnetic Nanoemulsions for Intra-Articular Therapy: Preclinical In Vitro and In Vivo Evaluation

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Abstract

Background. Osteoarthritis (OA) is a globally prevalent degenerative joint disease that significantly impairs quality of life and contributes to growing healthcare costs. Despite its impact, effective disease-modifying treatments are yet unavailable. Advanced drug delivery platforms offer a promising avenue to improve the precision and efficacy of both current and emerging OA therapies.

Objective. This study aimed to design and assess a multifunctional intra-articular therapeutic system composed of magnetic nanoparticles (MNPs) embedded in nanoemulsions (NEs) co-loaded with dexamethasone (Dex) and ascorbic acid (AA) for patient adaptable OA management.

Materials and Methods. The physicochemical characteristics and stability of the NE-MNP formulation were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), and Fourier-transform infrared spectroscopy (FTIR). Cytocompatibility was investigated via MTT assays and LIVE/DEAD staining. Functional in vitro assays—focused on, wound healing, and chondrogenic potential—of adipose-derived stem cells (ADSCs) exposed to NE-MNPs with Dex and AA. In vivo, systemic toxicity and biodistribution were examined after intraperitoneal administration in mice, while therapeutic outcomes were assessed via intra-articular injection in a rat model of collagenase-induced OA. Hematological parameters, CRP levels, and histological analyses of the liver, kidney, heart, lungs, spleen, and muscle were recorded. Magnetic resonance imaging (MRI) and joint histology were evaluated at one and three months post-treatment.

Results. The co-loaded NE-MNPs exhibited a particle size range of 20–60 nm, long-term colloidal stability, and resistance to aggregation. In vitro, the system significantly promoted chondrogenic differentiation in ADSCs and in vitro wound healing. In mice, systemic administration showed no hematological or immunological alterations, and histology revealed no chronic organ damage aside from transient Kupffer cell activation. Intra-

articular delivery in the rat OA model led to diminished synovial inflammation, , and increased cartilage thickness within one and three months of administration.

Discussion. The results demonstrate that the Dex- and AA-loaded NE-MNP platform is safe, biologically active, and capable of modulating key pathogenic mechanisms in OA. The dual-acting system enhanced cartilage regeneration and suppressed disease progression in both cellular and animal models. Further validation and mechanistic studies is warranted to support translation.

Conclusions. This study provides preclinical evidence that magnetic nanoemulsion carriers co-loaded with Dex and AA may represent a novel and effective approach to intra-articular OA therapy, with potential to alter disease trajectory.

Po1_3.01

Tribological evaluation of melt electrowritten three-layer PCL multi-scale scaffolds for articular cartilage repair

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Abstract

Introduction

Articular cartilage, with its unique hierarchical architecture, is capable of withstanding dynamic loads while reducing friction, but lacks self-repair capacity due to its avascular nature. Replicating this structure is a key step towards engineering scaffolds with similar mechanical and tribological properties to native cartilage. Melt electrowriting (MEW), with its precise control over fibre deposition, offers a promising approach for fabricating such scaffolds. However, the tribological performance of MEW poly(ϵ -caprolactone) (PCL) scaffolds remains largely unknown. This work explores the relationship between scaffold architecture and tribological performance in tri-layered MEW PCL constructs, providing valuable insights into the design and optimization of future engineered cartilage scaffolds.

Materials and Methods

Medical-grade PCL was processed using a custom-built MEW device to fabricate scaffolds with multi-scale, tri-layered architectures. Three distinct layers were created: a base grid with two fiber orientations (0°-90° [D1] and 0°-60°-120° [D2]), a middle layer comprising 0°-45°-90°-135° fibers, and a superficial nanomesh. Scaffold morphology was characterized using scanning electron microscopy (SEM) and profilometry. Tribological performance was assessed using a nanotribometer with a Delrin pin at sliding speeds of 1 mm/s and 10 mm/s in PBS. The coefficient of friction (COF) and surface fibrillation were analyzed.

Results

SEM analysis confirmed the successful fabrication of the tri-layered scaffold architecture, revealing distinct fiber diameters of approximately $18.1 \pm 0.8 \mu\text{m}$ for the base grid layer, $6.65 \pm 0.80 \mu\text{m}$ for the middle layer, and $0.75 \pm 0.69 \mu\text{m}$ for the surface nanomesh. While both D1 and D2 scaffolds exhibited reduced friction response (figure 1), visually similar to the behavior of natural cartilage, their COF exceeded those observed in native tissue (ca. 0.1). Higher sliding speeds (10 mm/s) led to increased surface fibrillation, evidenced by the formation of micro-cracks and rougher surface textures observed via SEM (figure 2). Differences in the COF profiles between D1 and D2 scaffolds suggest that fiber orientation influences tribology and wear.

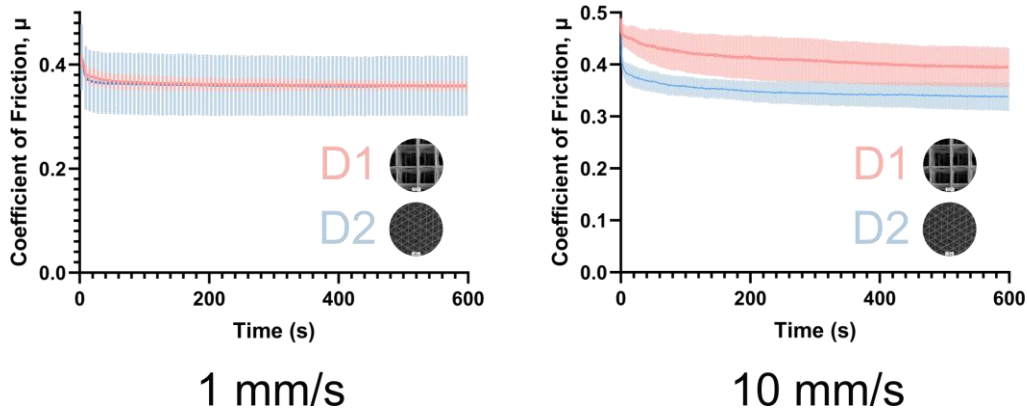


Figure 1: COF vs time

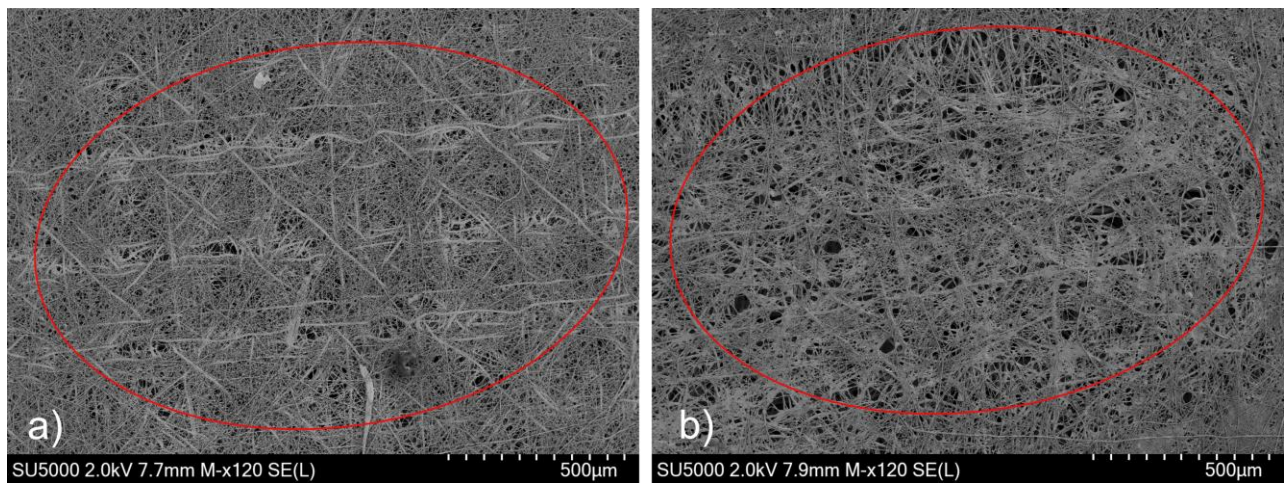


Figure 2: Post-test surface morphology at a) 1 mm/s and b) 10 mm/s

Conclusion

Tri-layered melt electrowritten PCL scaffolds demonstrated promising tribological properties under simplified dynamic loading. Observed fibrillation and friction behavior warrant further investigation under dynamic loading and long-term wear analysis. This study establishes a valuable evaluation protocol for complex MEW scaffolds.

Acknowledgments

This work is funded by the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie grant agreement No.956004

Po1_3.02

Multi-Ion Reinforced Collagen Hydrogels: A Therapeutic Strategy for Osteoporosis

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Abstract

Osteoporosis (OP) is a prevalent condition marked by increase in bone resorption, leading to a reduction in bone density. Multiple risk factors are associated with the development of OP, including low body weight, smoking, aging, nulliparity, sedentary lifestyle, as well as inadequate diets^{1,2}. Fractures resulting from OP significantly impact the quality of life of approximately 20 million individuals and place a considerable financial burden on healthcare systems, with annual costs reaching €37.5 billion³. The current used treatments confine the problem without resolving it and several side effects are reported^{4,5}. To address these limitations, this study is to develop an injectable biomimetic collagen hydrogel loaded with multiple-ions for bone repair.

An injectable collagen-based hydrogel was developed. Different ion precursors (e.g. iron) and nanoparticles (e.g. hydroxyapatite) were investigated to improve the biomechanical stability of the hydrogels. The ion precursors were introduced drop by drop into the homogenized collagen solutions. The material was transferred between two syringes connected syringes to ensure better homogenization during synthesis. The final hydrogels were poured into a small cylindrical plastic mould and submerged in a Dulbecco's phosphate buffered saline solution for one day. The chemical, mechanical and biological properties of the hydrogels were investigated.

The interaction between the injected precursor ions and the carboxylic groups of the collagen matrix was demonstrated by Fourier transform infrared spectroscopy. By adding these precursors to the collagen, a homogeneous hydrogel is formed, which improves the mechanical qualities of the material. In addition, the Live&Dead and Resazurin tests showed that MC3T3-E1 Subclone 4 pre-osteoblastic cells are not cytotoxically affected by the addition of precursor ions to collagen.

The incorporation of multiple ions into collagen hydrogels increases mechanical properties without inducing cytotoxicity. Additional tests will be carried out to select the most suitable condition to promote the hydrogel formation, as well as to be prompt as osteoporosis treatment.

Keywords: Injectable hydrogel; Osteoporosis; collagen; precursors ions; nanoparticles.

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Po1_3.03

Effect of different Polycaprolactone/Bioactive Glass composite scaffolds on the osteogenic potential of RIA-derived mesenchymal stem cells in a 3D cell culture

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Abstract

Background: Scaffold guided bone regeneration is an emerging and promising treatment method for large size bone defects. 3D-printed Polycaprolactone (PCL)/Bioactive Glass (BG) composite scaffolds combine inert and fully resorbable aspects of PCL with osteostimulative and antimicrobial properties of BG. In this study we evaluated the osteogenic potential of Reamer-Irrigator-Aspirator (RIA-)derived mesenchymal stem cells (MSC) colonized on PCL/BG scaffolds with different BG proportions in-vitro.

Methods: Cylindrical, porous 3D printed PCL/BG scaffolds (diameter 6mm, height 7mm) made out of PCL with different BG proportions (0, 10, 20, 30%) were analyzed (PCL, BG10, BG20, BG30) with n=12 scaffolds per group.

Human RIA-MSC were seeded with a density of 3 million cells/scaffold and cultured for 21 days.

For each group, 6 samples underwent osteogenic differentiation in DMEM with osteogenic factors (OM), while 6 samples functioned as control without osteogenic differentiation (C). On day 21, osteogenic potential was assessed by 99mTechnetium-Hydroxydiphosphonate (99mTc-HDP) labelling (incubation with 10 MBq Tc-HDP for 30min and measuring hydroxyapatite-bounded Tc-HDP after washing). Blank scaffolds were analyzed as well to rule out an uptake increase caused by direct Tc-HDP binding to BG. For statistical analysis, data were examined for normal distribution and ANOVA analysis with Bonferroni post-hoc testing was performed ($p \leq 0.05$).

Results: The BG30 OM group showed the highest Tc-HDP uptake (mean value: 1,49 MBq), statistically significant vs. BG10 OM (0,96 MBq, $p = 0,026$) and PCL OM (0,84MBq, $p = 0,002$). BG20 OM (1,35 MBq) showed a significantly higher uptake vs. PCL OM ($p = 0,038$).

Apparently the BG30 C uptake was 1,20 MBq, even without the presence of osteogenic factors, significantly higher vs. BG10 C (0,16MBq, $p < 0,001$) and PCL C (0,15MBq, $p < 0,001$).

Conclusions: Our data revealed that a higher amount of BG in the scaffolds led to a higher uptake of the radioactive tracer indicating that a high amount of BG is leading to osteogenic differentiation of cells, in this experiment RIA-derived MSCs, and formation of hydroxyapatite.

This effect can even be observed in the control group without osteogenic medium, which once again confirms the osteostimulative effect of BG in-vitro.

This Abstract presents a strong fundament for further in-vitro and in-vivo studies on the osteogenic potential of PCL/BG scaffolds. If further studies still show the high osteogenic potential of BG-containing scaffolds compared to pure PCL, PCL/BG scaffolds are a promising treatment option for large bone defects in humans.

Po1_3.05

Preclinical investigation of a novel constant force implant concept for the treatment of leg length discrepancies

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Abstract

Purpose Leg length discrepancies (LLD) in children and adolescent are often corrected by temporary epiphysiodesis, in which the growth of the longer leg is inhibited by an implant. With standard implant solutions, the acting forces cannot be controlled potentially leading to problems like mechanical failure or secondary deformities such as varus-valgus (Tirta et al., ActaOrthop, 2024) and tibia roof deformities (Tolk et al., StrategiesTraumaLimbReconstr, 2022). A novel implant concept was developed consisting of a cerclage wire with a twisted coil that unwinds with growth, exerting a controlled constant force to the physis (Buschbaum et al., JChildOrthop., 2021). The study aim was to proof the concept in a large animal experiment by evaluating the effect of three different force levels (200N, 120N, 60N) on the creation of LLD.

Methods The left proximal tibial physes of eighteen lambs were treated bilaterally with two implants and were assigned to three groups distinct by the implant force level. LLD was calculated by the length of the right and left tibiae measured on biweekly anteroposterior (AP) radiographs. Linear regression analysis was deployed to determine the reduction rates and the percentage of inhibited growth. Articular surface changes of the tibial plateau and the angular deformities were measured in AP radiographs post-operatively and at study end. CT scans were taken in 2-month intervals to confirm implant functionality.

Results The treatment appeared safe without implant related failures. The 200N implant achieved the highest growth inhibition (64%), whereas the 60N implant was less effective (13%). A medium effect was observed in the 120N group (36%). The average LLD was 9.3 mm (SD 3.1 mm), 5.7 mm (SD 1.1 mm) and 1.1 mm (SD 2.8 mm) for the 200N, 120N and 60N group respectively. Articular surface deformation was clearly visible in the 200N group with a change in the tibial roof angle of 3.6° (SD 1.8°). Marginal deformation was observed in the other groups (120N: 0.7°, SD 2.0°; 60N: 0.4°, SD 1.6°). No relevant varus-valgus-deformity was created in any group (200N: 1.00°, SD 2.4; 120N: 1.6°, SD 2.9; 60N: 1.3°, SD 1.1°).

Conclusion By means of the constant force concept controlled growth modulation appeared feasible in this preclinical experiment. Medium force implant (120N) appeared to be a reasonable compromise between force level and avoidance of articular surface deformation. However, clinical trials are necessary to confirm whether the results are translatable to the human pathologic situation.

Po1_3.06

Development and Preliminary Clinical Evaluation of a Novel Ankle Joint Prosthesis

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Abstract

Objective

To address issues such as prosthesis loosening and liner wear in current total ankle arthroplasty, a novel ankle joint prosthesis was developed and its performance and preliminary clinical outcomes were evaluated.

Methods

Materials and Manufacturing: A 3D-printed porous tantalum ankle joint prosthesis was fabricated using spherical tantalum powder (purity >99.95 wt%) via selective laser melting (SLM). The articular surface was polished, and a TiN ceramic coating was deposited via magnetron sputtering to enhance surface hardness and wear resistance.

Performance Testing: Scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) were employed to characterize the materials and coatings. Nanoindentation, scratch testing, and pin-on-disk tests were conducted to assess mechanical properties, adhesion strength, and tribological performance.

Clinical Translation: The prosthesis was applied to 10 patients with end-stage ankle osteoarthritis and talar necrosis. Preoperative and postoperative scores, along with complications, were recorded.

Results

The porous tantalum prosthesis was successfully fabricated with a porosity of 80% and pore size of 800 μm , exhibiting a clean and contamination-free surface. The TiN coating significantly improved surface hardness (from 9.39 GPa to 20.75 GPa) and elastic modulus (from 231.30 GPa to 354.73 GPa) while reducing the friction coefficient (from 0.87 to 0.2). Clinical outcomes showed that postoperative patient scores improved from a preoperative average of 53 to 89, with no significant complications.

Conclusion

The novel ankle joint prosthesis, featuring innovations in materials, structure, and interface design, demonstrated significantly enhanced mechanical performance and wear resistance. Preliminary clinical outcomes were favorable, providing a promising new solution for total ankle arthroplasty.

Po1_3.07

From 3D to 4D – The future of personalized 3D printed medical implants

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Abstract

The rapid advancement of additive manufacturing technologies has ushered in a new era of personalized orthopedic implants, allowing practitioners to tailor solutions precisely to patients' anatomical and physiological needs. Traditionally, 3D printed, single material titanium-based implants have seen extensive use due to their exceptional biocompatibility, strength, and osseointegration properties. However, these static constructs are inherently limited in two key areas: One is their much higher rigidity, in comparison with the surrounding bone, which creates stress shielding on bone-implant interfaces (rigid metal pushing against elastic bone), and leads to loosening of the implant over time. The other is their inability to adapt over time, to change, creating a demand for the next generation of "4D" implants—materials that transform, remodel, or degrade as the patient's body heals and grows.

This presentation will explore the concept of transitioning from purely three-dimensional titanium implants toward multi-material, bi-metallic constructs that incorporate slow-resorbable components. By introducing carefully selected degradable materials into titanium-based lattices, we can engineer implants that gradually change their mechanical and structural attributes. Unlike conventional permanent hardware, these hybrid constructs are designed to evolve, maintaining structural integrity where needed, while enabling better osteointegration on a macroscopic level, eventually providing a seamless interface between implant and bone.

In particular, we will highlight novel manufacturing techniques that merge titanium alloys with biodegradable metals or ceramics, creating functionally graded structures that demonstrate controlled corrosion profiles and tailored mechanical properties. Using advanced laser-based additive manufacturing methods, it is possible to create complex geometries, through which we can distribute distinct materials to encourage site-specific degradation and tissue ingrowth. This approach not only mitigates stress shielding and implant-related complications, but it also paves the way for a patient-specific, time-dependent healing environment.

To illustrate this future, the presentation will showcase preliminary results of manufacturing possibilities and geometric concepts. We will also discuss the critical challenges of this emerging field, including the optimization of multi-material interfaces, long-term biocompatibility, and the establishment of standardized methods for predicting degradation kinetics and mechanical performance. Ultimately, moving "From 3D to 4D" is poised to revolutionize how we conceive and fabricate medical implants, setting a new standard for dynamic, patient-specific, and biologically integrated orthopedic solutions.

Po1_3.08

Pre-clinical biomechanical assessment of a patient-matched multi-parametric osseointegrated prosthesis for transfemoral amputees

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Abstract

Introduction: Osseointegrated prostheses for transfemoral amputees are a viable alternative to socket ones. In fact, osseointegration provides better control over the prosthesis, while overcoming the limitations of socket and suspension systems, which lead to a significant abandon rate. Osseointegrated systems are currently available in discrete sizes, providing a limited options in terms of diameter, and sometimes length and curvature. We recently developed a patient-matched multi-parametric osseointegrated system (OsteoCustom) to match the anatomy of the host bone not only in terms of diameter, but also conicity, ellipticity, and curvature. In this paper we present the extensive pre-clinical validation we performed.

Fit-and-fill: To quantify the benefits of the OsteoCustom stem on the fit-and-fill, CT scans of 70 femurs were virtually implanted with the OsteoCustom stem and with a commercial one (OFI-C). The OsteoCustom could be implanted in all femurs, while the OFI-C was unsuitable in 19 cases due to insufficient cortical thickness. The OsteoCustom stem preserved significant volume of cortical bone compared to the OFI-C (33%-42% bone spared, $p \leq 0.0001$). The stem-bone contact area was greater for the OsteoCustom, particularly distally (20% increase, $p = 0.002$).

Endurance: To ensure adequate mechanical endurance of the stem, a fatigue test was designed, based on the loads in the literature. As it is not affordable to perform fatigue tests on each design combination, a workflow was developed where finite element (FE) models are used. To prove the credibility of the FE models, dedicated experiments were performed, where the strains and displacements of the stem were measured at the most relevant locations. These FE model can therefore reliably “test” the mechanical strength of each OsteoCustom stem.

Implant stability and load transfer: As stem loosening and adverse bone remodelling are concerns for osseointegrated implants, biomechanical tests were performed on 9 femur pairs. One femur of each pair was implanted with OsteoCustom. Only 6 of the 9 contralateral ones could receive an OFI-C stem due to anatomical mismatch. To test implant stability and load-transfer, each implant was subjected to cyclic compression-bending and torsional tests. Implant micromotions and bone strains were measured with a

digital image correlation (DIC) system. The inducible micromotions of the OsteoCustom were 3-4 times smaller than the OFI-C ($p < 0.05$). The strain distribution of the OFI-C showed slightly higher strain-concentrations near the stem tip than the OsteoCustom.

Conclusions: These findings provide a solid ground for starting a clinical trial on the OsteoCustom.

Acknowledgements: “OsteoAMI” project funded by INAIL (PR23-CR-P5).

Po1_3.09

Mid-term Analysis of the Survival of Uncemented Total Knee Arthroplasties with Modular Tibial Components Made of Porous Tantalum

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Abstract

Introduction: Uncemented total knee arthroplasties (TKAs) were designed to reduce the revision rate due to aseptic loosening, particularly in young, active males with osteoarthritis (OA), who have higher demands on their knee joints. This retrospective study aimed to compare the survival rates of uncemented TKAs with porous tantalum posterior-stabilized (PS) modular tibial components to those with cemented PS tibial components from the same knee implant system by the same manufacturer, using revision for aseptic loosening as the primary endpoint.

Materials and Methods: From January 2017 to December 2021, 1,202 TKAs were performed at a single tertiary institution by five experienced orthopaedic surgeons. The majority of patients underwent TKA for primary OA. Of the total cohort, 826 cases (68.7%) involved cemented TKAs, while 376 cases (31.3%) involved uncemented TKAs. The distribution of body mass index (BMI) was similar between both groups (mean BMI: 30.9 kg/m², $p > 0.05$). However, patients treated with uncemented TKAs were younger (mean age 64.4 years, 95% CI 63.6-65.2) than those with cemented TKAs (mean age 70.6 years, 95% CI 70.1-71.2) and were predominantly male (59%), while the cemented group had a higher proportion of female patients (74.2%). The cohort was followed until December 31, 2023.

Po1_3.11

Patient-specific Distal Tibia Titanium Trabecular prosthesis for reconstruction in a case of recurrent Giant Cell Tumour in a Young female. 2-year retrospective Case study

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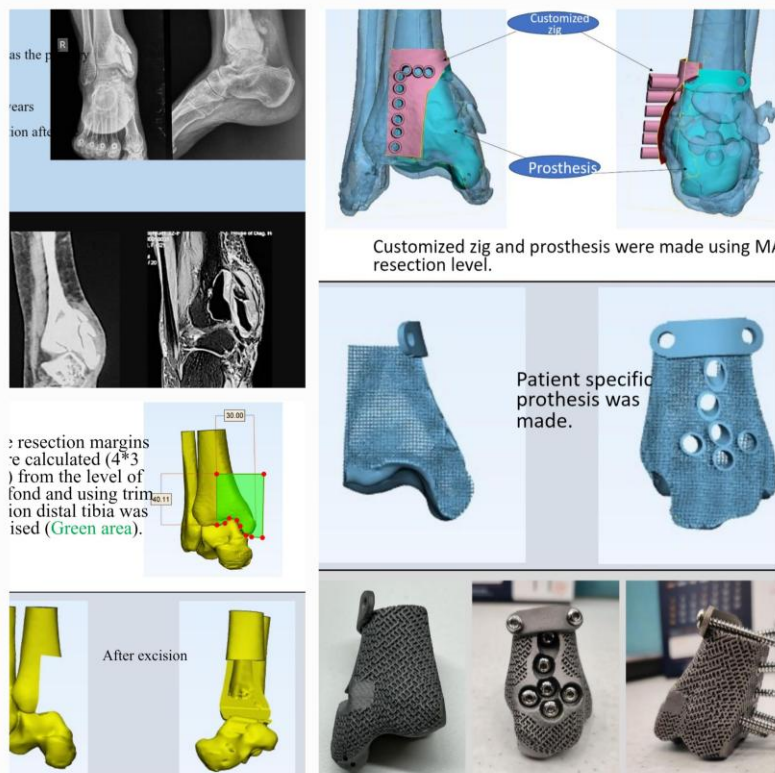
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Abstract

Keywords: 3-D printed titanium trabeculae prosthetic implant, Osteointegration, Giant Cell Tumour, Ankle Joint preservation, metal on cartilage bearing.

Introduction: Giant cell tumours (GCT) of bone presenting in the distal tibia are rare, however, when they occur, they can present a reconstructive challenge. **Shortening and fusion of joints** cause disability.

They are associated with a high local reoccurrence rate with intralesional treatment, therefore approaches to reconstruct the ankle with metal anatomical implant after en-bloc resection warrant study.

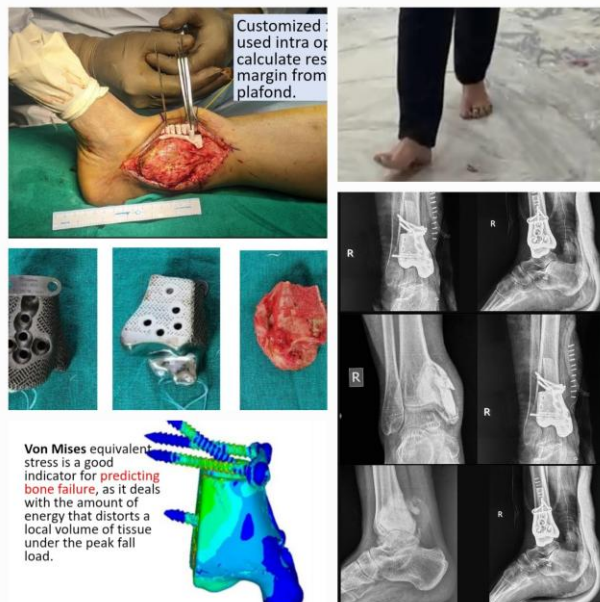


The primary objective of this Abstract is to share the experience of developing a *patient-specific customized distal tibial prosthesis* to reconstruct a resection gap (defect) in a recurrent giant cell tumour. The implant was designed and metal printed using 3D technology with the aim of Joint preservation and *osteointegration* of metal to bone and *metal on cartilage* articulation.

We describe a case of a 32-year-old female with a Recurrence of GCT of Distal tibia. She was treated initially with curettage and cementing as the primary treatment and had a recurrence after 4 years when she presented to us. The patient underwent a biopsy; CT, and MRI imaging for diagnosis and the extent of the tumour. She was planned for resection and reconstruction after pre-surgical Denosumab.

Pre-operative planning was done using CT scan data on definitive planning software. The resection gap was precisely calculated. The calculated gap was mirrored over the opposite limb. The model was created, it was meshed with specific pore size, and modified to create intramedullary space for bone grafting, and holes for fixation. This virtual model was finally printed on a Metal 3D printer. The finalized model was printed with titanium metal **Ti-6Al-4V** and the interface with bone was coated with hydroxyl-apatite. The joint articulating surface was polished and mirrored to create absolute congruency and keep the coefficient of friction less than 0.002. Definite Design holes were made for soft tissue attachments.

The patient-specific customized titanium implant prosthesis was implanted after resection of the tumour as planned. The result was satisfactory, at 24 months of follow-up up there was no recurrence and the patient had a full weight-bearing ability, pain-free and nearly full ROM at the ankle joint. Radiologically the implant is integrated well with the bone.



Po1_3.12

Impact of Cyclic Loading on the Mechanical Properties of Degradable Braided Scaffolds for ACL Reconstruction: For Walking and Running Conditions

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Abstract

Purpose: This study investigates the mechanical performance of braided poly-ε-caprolactone (PCL) scaffolds under cyclic loading in physiological gait conditions to assess their potential as Anterior Cruciate Ligament (ACL) implants as a tissue engineering approach.

Introduction: One of the key limitations of ACL reconstruction is the availability of suitable grafts. Braided degradable scaffolds have been engineered as a synthetic solution for ligament reconstruction, providing structural support and promoting tissue remodeling through cellular ingrowth. [1, 2] To ensure graft viability after surgery, its mechanical properties must be evaluated under physiological loading conditions. According to the literature, ligament devices should exhibit a maximum creep of 3-5mm to prevent laxity. [3, 4]

Methods: Braided PCL scaffolds (n=3) were fabricated from melt-spun PCL monofilaments. [5] The braids were designed to replicate a native ACL for ligament replacement and regeneration, with previous static tests confirming that their stiffness, tensile strength and degradation behavior match those of a native ACL. [6, 7] The scaffolds are tested under cyclic conditions with loads simulating running and walking to assess post-operative performance. The testing protocol involved a preload of 30N for 1 minute, followed by preconditioning (50 cycles at 1Hz, 0N–50N), simulated gait (250000 cycles at 1Hz, walking: 30N–210N, and at 1.5Hz for running: 30N–630N), and postconditioning (50 cycles at 1Hz, 0N–50N) [4, 8, 9]. All tests used a standardized 40mm gauge length.

Results: All scaffolds underwent cyclic testing, with none showing failure. During cyclic testing the scaffolds exhibit a maximal elongation up to 2.9mm for simulated walking and up to 8.78mm for simulated running. A comparison of the change in length between pre- and postconditioning shows a plastic deformation in a range of 1.46mm to 1.74mm for walking and 4.77mm to 5.69mm for running.

Conclusion: None of the braided PCL scaffolds failed during cyclic testing. The requirement of less than 5mm change in length is also fulfilled for simulated walking. Elongation during

walking ranged from 5.975% to 7.25%, consistent with native ACL's in vivo elongation of the anterior medial bundle $5.0 \pm 1.7\%$ and posterior anterior bundle $3.4 \pm 2.3\%$. [10] Under the extreme running conditions tested, which are not expected postoperatively, high elongation occurs, surpassing the native ACL range. Further investigation of the mechanical properties after cell ingrowth must still be tested for scaffold suitability under both conditions. With strong performance already demonstrated under walking conditions, the braid shows promising potential for scaffold applications.

Po1_3.13

First measurements of the Active Implant system based on SMA actuation and sensing for improved bone fracture healing.

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Abstract

Nickel-titanium-based thermal shape memory alloys (SMAs) contract at a specific temperature due to a reversible phase transformation (PT) from martensite to austenite and vice versa [1]. SMA technology is well-established in medicine due to its superelastic and biocompatible properties, especially in stents and guidewires [2]. Because of their versatile geometry, low weight, high energy density, and low cost [3]–[5], the use of SMAs as actuators is gaining popularity in the fields of industrial, aerospace, and automotive applications [6]–[9]. Recently, the arrangement of bundled micro-wires down to a wire diameter of 25 µm presents a continuous actuation frequency of 200 Hz [10], [11]. A further advantage of SMAs is their *self-sensing* capability [12], [13]: By measuring the electrical resistance of the SMA, the degree of PT and, thus, the contraction and applied force are monitored.

Recent scientific research found that a switchable stiffness of an implant for bone fracture treatment with individual stimulation patterns to be applied to the fracture site improves and accelerates the patient's healing process [14], [15]

The first innovative implant system, patent-pending [16], was developed and built as a proof of concept using aluminum [17], [18]. SMA actuators implement a switching capability between *rigid* and *soft* states. In the *soft* state, a second set of SMA actuators allows for controlled stimulation of the fracture site by contracting and restoring with an adaptable stroke of 0.3 mm.

This proof of concept has recently been equipped with a miniaturized control unit (CU) and a user interface (UI). [19] Due to the small size of the developed electronic with integrated battery and inductive charging, the CU is ready for test at the animal trial. The CU supports fast data acquisition, map-based control, and neural networks for fall risk assessment. The UI enables the control of the implant via a WebApp, using Bluetooth LE.

This work presents a novel integrated actuation design, which enables continuously variable adaption of the applied stroke and stiffness. [20] The design of the new implant demonstrator exhibits, in contrast to the proof of concept, a media-tight surface without moving parts on the surface, enabling the load-bearing capacity of a human body.

Along with the design, this work presents and discusses the initial measurement results from the dynamic load test. These experiments were conducted in a laboratory environment, where the new implant demonstrator is attached to a replica of human bone using standardized fixed-angle screws.

Po1_3.14

Experimental Study on the Design of a Biomimetic Osteochondral Scaffold Based on Microstructure Characteristics of the Adult Knee Joint Osteochondral Complex

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Abstract

Objective: To explore the microstructure characteristics of normal adult osteochondral complexes and provide a new design scheme for a bionic osteochondral scaffold model. **Methods:** Three adult cadavers without bone disease were randomly selected. Samples of uniform size were taken from the lateral condyle, medial condyle, and patellar surface of the femoral articular surface of the left and right knee joints. High-resolution micro-CT tomography was used to scan the specimens and measure the average thickness of cartilage and subchondral bone plate, as well as the morphometric parameters of trabecular bone (BV/TV, TbN, TbTh, TbSp). Based on the obtained anatomical data, the VG Studio MAX 3.4 software was used to construct an osteochondral scaffold model with specific internal structure. **Results:** The average thickness of the patellar cartilage was significantly greater than that of the lateral and medial condyles ($P < 0.05$). The average thickness of the patellar subchondral bone plate was greater than that of the lateral and medial condyles ($P < 0.05$). The thickness ratio of patellar cartilage to bone plate was significantly lower than that of the lateral and medial condyles ($P < 0.05$). The trabecular morphometric parameters TbSp and TbTh of the cancellous bone space showed significant differences between the lateral condyle and patellar surface ($P < 0.05$). Using VG Studio MAX 3.4, digital models for non-degradable and degradable materials were constructed, replicating the three-dimensional structure of the normal osteochondral complex. **Conclusion:** There are distinct microstructural differences in the osteochondral complex across different regions of the human knee joint. Based on Micro-CT data and computer-aided design, a digital 3D model of the osteochondral scaffold can be accurately constructed.

Po1_3.16

Collagen-Based 3D Scaffolds Enriched with Multi-Ion Doped Hydroxyapatite for Osteosarcoma Therapy

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Abstract

The limited ability of conventional two-dimensional (2D) tumor models to replicate the complexity of the osteosarcoma microenvironment—encompassing cellular interactions, tumor heterogeneity, and drug resistance—hinders advancements in cancer research and therapy development¹. This study introduces an innovative three-dimensional (3D) collagen-based scaffold designed to simultaneously support the removal of cancer cells and promote tissue regeneration, providing a biologically relevant platform for tumor modeling and therapeutic evaluation.

The scaffolds were fabricated using Viscolma® (a certified medical-grade collagen) and doped with multi-ion hydroxyapatite to mimic the mineralized matrix of osteosarcoma tissue. Collagen concentrations of 5% and 19% (w/v) were optimized for 3D printing via a custom piston-driven printer. A biomineralization process induced through ion baths resulted in scaffolds with various compositions: hydroxyapatite/collagen (ColHA), magnesium-doped hydroxyapatite/collagen (ColHAMg), iron-doped hydroxyapatite/collagen (ColFeHA), and iron/magnesium-doped hydroxyapatite/collagen (ColFeHAMg). Pure collagen scaffolds served as controls.

Comprehensive characterization was performed to evaluate the scaffolds' properties. Scanning electron microscopy (SEM) revealed a porous architecture with hydroxyapatite aggregates embedded in collagen fibers, while energy-dispersive X-ray spectroscopy (EDX) confirmed heterogeneous mineral distribution. Fourier transform infrared spectroscopy (FTIR) demonstrated strong chemical interactions between collagen and hydroxyapatite,

and thermogravimetric analysis (TGA) highlighted the stability of the mineral phase during collagen degradation. Notably, 5% collagen scaffolds exhibited superior structural stability and degradation resistance, enhancing their suitability for long-term applications.

This innovative hybrid scaffold replicates the compositional and structural complexity of mineralized osteosarcoma tissue, establishing a dual-functional platform for cancer cell targeting and tissue regeneration. Its potential to closely emulate the tumor microenvironment offers a transformative approach for studying osteosarcoma biology, evaluating anticancer strategies, and accelerating the translation of tissue-engineered solutions into clinical practice.

Keywords: Bio-inspired mineralization; 3D tumor models; Functional gradient structures; Osteosarcoma microenvironment

Reference

1. <https://doi.org/10.1016/j.ctarc.2021.100324>

Po1_3.17

Dual-Polymer Mineralized Hydroxyapatite Scaffolds for Enhanced Bone Tissue Regeneration

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Abstract

Bone tissue lesions represent a significant public health challenge, with biomaterial-based therapies emerging as effective and safer alternatives¹. Bone is primarily composed of calcium phosphate, type I collagen, non-collagenous proteins, growth factors, water, and cells². However, current treatment strategies for bone defects often demonstrate limited efficacy³. As a result, research efforts have been directed toward developing scaffolds capable of filling voids caused by substantial bone loss, thereby promoting regeneration⁴. Collagen, an autologous component of the extracellular matrix, has been widely utilized for scaffold fabrication⁵. Despite its biocompatibility, collagen exhibits limitations such as high degradability and insufficient mechanical strength. To address these challenges, a bio-inspired mineralization process was employed to create hybrid materials. This approach involved the heterogeneous nucleation of hydroxyapatite nanocrystals on collagen nanofibers, which were subsequently engineered into 3D-printed scaffolds. These novel constructs hold significant potential for advancing bone tissue regeneration. The biomineralization process was utilized to synthesize a hybrid material comprising collagen mineralized with hydroxyapatite. Various synthesis and printing parameters were explored, and the scaffolds were fabricated using a customized biofabrication system. Were developed two-layer scaffolds with a 0/90° geometry. Then, the chemical properties, injectability, morphology, and biological performance of the scaffolds were thoroughly evaluated. Fabrication was conducted at a deposition velocity of 10 mm/s, although inconsistencies in the slurry composition prevented extrusion in some samples. Both pore and filament sizes deviated from theoretical values. The findings demonstrated that the Food Printer system successfully produced scaffolds with diverse formulations and the intended 0/90° geometry. Chemical analysis revealed interactions between collagen molecules and the mineral phase⁶. Synthesis conditions influenced collagen fiber formation

and material injectability, as evidenced by morphological and rheological assessments. Biological tests indicated that all scaffold formulations supported the adhesion and proliferation of human mesenchymal stem cells (hMSCs). Collagen-doped hydroxyapatite scaffolds were successfully fabricated via 3D printing.

Further optimization is needed to achieve an ideal balance between mechanical strength and scaffold geometry. These scaffolds hold significant promise for advancing bone tissue repair and regeneration.

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Keywords: Biofabrication, Biomimicry; biomineralization process

Po1_3.19

Investigation of Zinc-, Copper-, and Silver-Doped Fluorapatite as Bacteriostatic Bone Grafts

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Abstract

Objective: Synthetic bone substitutes are increasingly being investigated as graft materials for dental and craniofacial reconstructive surgeries, where autografts and allografts xenografts are standard but are limited by high failure rates. Infection is one of the most common causes of graft failure, as it can impede healing and prevent proper integration with bone tissue, resulting in excessive cost and patient mobilities. Bacterial contamination during surgery or inadequate post-operative care can increase the risk of infection, which is more common in the contaminated oral cavity. Our previous study demonstrated that fluorapatite (FA), a fluorine-substituted form of hydroxyapatite (HA), enhances bone formation and performs comparably to autografts in *in vivo* models. Additionally, the literature suggests that metal doping can improve the antimicrobial properties of HA. However, limited studies have explored metal-doped FA's antimicrobial efficacies. We hypothesized that low levels of metal doping could enhance the antimicrobial properties of FA. To test this, we doped FA with 0.25% to 10% zinc, copper, or silver—known antimicrobial metals—characterized them, evaluated the bacteriostatic properties of these metal-doped FA surfaces, and tested the cytotoxicity in osteoblast cell cultures.

Methods: Metal-doped FA was initially synthesized and calcined at 900°C and then characterized using X-ray diffraction, Fourier-transform infrared spectroscopy, and inductively coupled plasma mass spectrometry. Discs were fabricated and sintered at 1150°C, and their surface morphology and topography were analyzed using scanning electron microscopy and energy-dispersive X-ray spectroscopy. The bacteriostatic properties of these discs were evaluated after a 2-hour incubation with *Staphylococcus aureus*, followed by serial dilution and the spread plate method. Additionally, cytotoxicity studies were performed on selected surfaces with antimicrobial properties using osteoblasts.

Results and Discussion: Characterization data revealed the presence of metal oxides in the highly doped apatite compositions, while substitution levels of 0.25% to 1% did not result in the formation of oxides. Scanning electron microscopy showed distinct surface morphologies that varied depending on the percentage and type of metal doping. Except for silver-doped FA, surface grain sizes increased with higher percentages of dopant metals, creating micro-topographies. Compared to undoped FA, 0.25% to 5% copper-doped FA

exhibited a ~2-3 log-fold reduction in bacterial adhesion, rendering them bacteriostatic surfaces. Osteoblast cell culture studies showed no cytotoxicity when copper was doped at less than 1%. These low-percentage copper-doped FA materials have the potential as ideal synthetic bone substitutes for contaminated sites, pending further validation *in vivo* models.

Po1_3.20

Multidisciplinary optimal design for improved personalized bioactive glass/ceramic bone substitute implants

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Abstract

INTRODUCTION:

Clinical challenges often overload healthcare systems, particularly in cases involving the musculoskeletal system, which is highly vulnerable to aging and trauma. To address the significant need for bone-substitute implants, new approaches are essential. These solutions should include personalized treatments for improved patient outcomes, advancements in materials for greater mechanical durability without compromising bioactivity or bioresorbability, and improved manufacturing methods to ensure high-quality and reliable products.

In this presentation the approach pursued with the ReBone Doctoral Network (<https://rebone.eu/>), a four-year program funded by the Europe Horizon Marie Skłodowska-Curie initiative, will be shown. This program aims to optimize technologies for creating customized bone-substitute implants using bioactive ceramics and advanced additive manufacturing techniques, thereby tackling the health and societal challenges posed by trauma and bone diseases.

MATERIALS and METHODS: The program establishes a multidisciplinary network and training framework where ten doctoral candidates will collaboratively create an innovative methodology for designing ceramic-based, personalized bone substitute implants. The network, encompassing partners across Europe, integrates expertise in biomechanics, clinical applications, materials engineering, mechanobiology, additive manufacturing, and mixed-reality surgical planning.

Materials are a central focus, emphasizing mechanical properties, bioactivity, biocompatibility, and printing precision. Experts, including biologists, materials engineers, bioengineers, and technology developers, will work together to develop and thoroughly test glass-ceramic-based materials for these implants. Real-case scenarios will be derived from four selected clinical cases requiring bone repair, enabling the creation of personalized, multi-scale implant models. These models will integrate clinical data, optimizing the implant's architecture, material properties, and manufacturing parameters to deliver improved mechanical performance tailored to the anatomical location and shape of the implant.

RESULTS AND DISCUSSION: In the proposed presentation, a general overview of the approach pursued in the project and preliminary results achieved will be shown, with particular reference, but not limited to, the design strategies and material selections adopted in the project.

CONCLUSIONS: The ReBone Doctoral Network, supported by the Europe Horizon Marie Skłodowska-Curie initiative, seeks to resolve challenges related to bone-substitute implants for critical-sized bone defects. This multidisciplinary effort prioritizes technological and material innovations as the basis for designing personalized solutions that address these pressing clinical needs.

Po1_3.24

Application of 3D printing Integrated Acetabular Prosthesis in the treatment of hip dysplasia in total hip arthroplasty

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Abstract

Objective: To investigate the clinical efficacy of 3D printed integrated acetabular prosthesis (IAP) in total hip arthroplasty (THA) of developmental dysplasia of the hip (DDH) with different Crowe types.

Methods: A total of 85 patients (106 hips) with DDH who underwent THA in the Department of Orthopedics, Affiliated Zhongshan Hospital of Dalian University from February 2020 to February 2022 were retrospectively analyzed. There were 48 cases (56 hips) of Crowe type I, 10 cases (14 hips) of Crowe type II, 15 cases (18 hips) of Crowe type III, and 12 cases (18 hips) of Crowe type IV. A series of IAP were designed and printed for each Crowe types. The hip joint was reconstructed according to the pre-operative design. The bilateral horizontal center of rotation (H-COR) difference and the bilateral vertical center of rotation (V-COR) difference before and after operation were measured and compared. Measure cup inclination and anteversion angle. The Harris hip score (HHS) was used to evaluate the hip function at 3 months after operation and at the last follow-up. The short form 36 questionnaire (SF-36) was used to evaluate the quality of life at 3 months after operation and at the last follow-up.

Results: All the operations were successfully completed, and the postoperative follow-up time was 26.8 ± 3.4 months. The bilateral V-COR difference decreased from 14.5 ± 13.8 mm to 3.8 ± 3.7 mm, and the bilateral H-COR difference decreased from 11.4 ± 7.7 mm to 2.0 ± 2.2 mm. The cup inclination and anteversion angle have been satisfactorily restored. The HHS was 82.6 ± 5.7 points at 3 months after operation and 90.4 ± 3.3 points at the last follow-up. The SF-36 was 625 ± 45 points at 3 months after operation and 748 ± 32 points at the last follow-up. No complications such as hip periprosthetic infection, prosthesis dislocation, or aseptic loosening occurred.

Conclusion: The use of 3D printed porous tantalum IAP in THA in the treatment of various types of DDH can fully ensure the acetabulum's coverage, accurately restoring the rotation center of the hip, which is helpful for the recovery of hip joint function. The early clinical efficacy is satisfactory.

Po1_3.26

HOBbit - Highly porous bioinert bone implants for non-union fracture healing

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Abstract

Background: Non-union fractures fail to heal on their own. The current gold standard, treatment with autologous bone grafts from the iliac crest, is difficult and often not possible. HOBbit aimed to develop a novel type of macro-porous implants that are based on high-strength, biocompatible ceramics as a treatment alternative. The goal of this project was to investigate the potential of zirconia (ZrO₂) and alumina (Al₂O₃) as substrates for minimal-volume high-performance ceramics to serve as load-bearing scaffolds and to examine the bone healing potential of a minimal volume, load-bearing scaffold combined with a gene-activated matrix, using either non-viral gene delivery and mesenchymal stromal cells (MSCs) or extracellular vesicles.

Methods: Ceramics were tested for biocompatibility in vitro and for mechanical stability for in vivo application. Load-bearing and regenerative potential of ceramics were evaluated in a rat femur critical size defect model of 5mm. To improve bone regeneration and prevent soft tissue ingrowth silk fibroin tubes were introduced. μ CT scans were performed after 3d, 2w, 4w, 6w and 8w for Al₂O₃. Hard tissue histology was performed after 8w for Al₂O₃ and ZrO₂.

Results: Both ceramics did not present toxicity in vitro and showed high mechanical performance. However, Al₂O₃ scaffolds did not provide sufficient load-bearing in vivo, resulting in breaking during the 8w observation period. ZrO₂ implants facilitated stability in the defect, and soft tissue ingrowth was prevented with silk fibroin tubes to allow bone formation through the whole scaffold.

Conclusion: Alterations in design and ceramic material improved stability and regeneration in the rat femur defect significantly over the 8W observation period. However, our results demonstrate the need for further elucidation regarding the introduction of a pro-regenerative matrix within the scaffolds to potentially enhance bone regeneration of patients.

Po1_4.01

Functional Outcomes and Imaging-Related Factors in Distal Radius Fractures Among Older Adults: A Comprehensive Review

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Abstract

Background:

Distal radius fractures (DRFs) are common in older adults, often resulting in significant functional impairments and reduced quality of life. Radiographic parameters such as dorsal/volar angulation, radial inclination, radial height, ulnar variance, and articular incongruity are emphasized in treatment planning, yet their relationship with functional recovery remains inconsistent. This systematic review synthesizes evidence from 23 studies to evaluate the associations between imaging-related factors and functional outcomes in elderly DRF patients, aiming to inform strategies that prioritize functionality over strict radiological alignment.

Methods:

A review of 23 studies was conducted, analyzing radiographic parameters—dorsal/volar angulation, radial inclination/height, ulnar variance, and articular incongruity—and their relationship with functional recovery. Outcomes were assessed using metrics such as the Disabilities of the Arm, Shoulder, and Hand (DASH) score, the Patient-Rated Wrist Evaluation (PRWE), grip strength, and range of motion. Treatment approaches, including surgical (e.g., volar locking plate fixation) and conservative (e.g., casting) methods, were evaluated for their effectiveness.

Results:

Radial inclination/height (14/23 studies), dorsal/volar angulation (10/23), and ulnar variance (10/23) were the most frequently studied radiographic factors. Surgical interventions improved radiological alignment but did not consistently correlate with better functional outcomes. Radial shortening and articular step-off were associated with worse outcomes, while radial inclination and ulnar variance correlated with improved recovery. Grip strength and range of motion emerged as stronger predictors of functional recovery than radiographic alignment, underscoring the importance of patient-centered approaches. Variability in findings highlights the influence of individual factors like age, comorbidities, and activity levels.

Conclusion:

Radiological parameters have limited predictive value for functional recovery in elderly DRF patients. Management should prioritize functionality, grip strength, and range of motion over radiographic alignment. Personalized protocols are essential to address the unique needs of older adults and improve long-term outcomes.

Po2_1.01

Application of 3D printing Integrated Acetabular Prosthesis in the treatment of hip dysplasia in total hip arthroplasty

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Abstract

To investigate the clinical efficacy of 3D printed integrated acetabular prosthesis (IAP) in total hip arthroplasty (THA) of developmental dysplasia of the hip (DDH) with different Crowe types.

A total of 85 patients (106 hips) with DDH who underwent THA in the Department of Orthopedics, Affiliated Zhongshan Hospital of Dalian University from February 2020 to February 2022 were retrospectively analyzed. There were 48 cases (56 hips) of Crowe type I, 10 cases (14 hips) of Crowe type II, 15 cases (18 hips) of Crowe type III, and 12 cases (18 hips) of Crowe type IV. A series of IAP were designed and printed for each Crowe types. The hip joint was reconstructed according to the pre-operative design. The bilateral horizontal center of rotation (H-COR) difference and the bilateral vertical center of rotation (V-COR) difference before and after operation were measured and compared. Measure cup inclination and anteversion angle. The Harris hip score (HHS) was used to evaluate the hip function at 3 months after operation and at the last follow-up.

All the operations were successfully completed, and the postoperative follow-up time was 26.8 ± 3.4 months. The bilateral V-COR difference decreased from 14.5 ± 13.8 mm to 3.8 ± 3.7 mm, and the bilateral H-COR difference decreased from 11.4 ± 7.7 mm to 2.0 ± 2.2 mm. The cup inclination and anteversion angle have been satisfactorily restored. The HHS was 82.6 ± 5.7 points at 3 months after operation and 90.4 ± 3.3 points at the last follow-up. No complications such as hip periprosthetic infection, prosthesis dislocation, or aseptic loosening occurred.

The use of 3D printed porous tantalum IAP in THA in the treatment of various types of DDH can fully ensure the acetabulum's coverage, accurately restoring the rotation center of the hip, which is helpful for the recovery of hip joint function. The early clinical efficacy is satisfactory.

Po2_1.06

Tailor-made: 3D Printing-assisted Autologous Costal Cartilage Remodeling in the Treatment of Complex Carpal Bone Lesions

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Abstract

Complex carpal bone lesions, such as advanced lunate necrosis (Kienböck's disease) and proximal pole necrosis of the scaphoid after scaphoid fracture, pose great difficulties in clinical treatment, and currently, there is no satisfactory treatment method. Based on 3D printing and digital technology, we have established a brand-new and precise costal cartilage transplantation surgical technique.

Preoperatively, the morphology of the necrotic lunate (or the proximal pole of the scaphoid) was accurately evaluated using CT images to determine its spatial parameters. Subsequently, the healthy lunate (or scaphoid) on the contralateral side was mirror-flipped, and a prosthesis (a complete lunate or the planned proximal pole of the scaphoid) that could be used for actual intraoperative evaluation was 3D printed. Using this as a template, the obtained autologous costal cartilage was manually carved into the shape of the lunate (or part of the scaphoid) required to fill the defect. After the surgical resection of the diseased carpal bones, the remodeled costal cartilage was transplanted and substituted into the carpal bone defect area (Figure1). The lunate was fixed by ligament transverse fixation, and the scaphoid was fixed by absorbable screw cross fixation. Postoperatively, the improvement of wrist joint pain, the range of joint motion, the quality of life score, as well as X-ray, CT and MRI were regularly followed up.

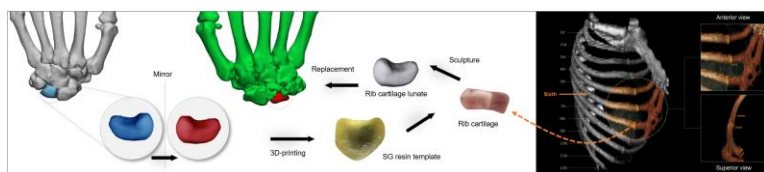


Figure 1. Surgical design and procedures

The clinical follow-up results for up to five years demonstrated that this surgical method could effectively relieve the wrist joint pain symptoms of patients, enhance the wrist joint function, significantly increase the range of wrist joint motion and grip strength, and effectively improve the quality of life. The postoperative follow-up X-ray, CT and MRI images showed that the grafts were in place, and no obvious ossification and absorption phenomena were observed (Figure2).



Figure 1. Surgical design and procedures

In conclusion, the precise remodeling and transplantation of autologous costal cartilage assisted by 3D printing can achieve accurate replacement for the necrotic lunate and the proximal pole of the scaphoid, representing a promising clinical treatment method for complex carpal bone lesions.

Po2_1.07

Reliability Evaluation of Surface-Based Registration Approach to Determine Femoral Rotation Using 3D Bone Models

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Abstract

The use of surface points from Three-dimensional (3D) bone models, as opposed to traditional landmark-based Two-dimensional (2D) methods, has gained significant interest in many orthopedic applications. This study applies this principle to quantify rotational changes in long bones before and after intervention, where here 'intervention' refers to the natural growth process between two time points. The proposed method's consistency and level of agreement with a conventional 2D measurement technique were assessed.

3D triangular surface models were reconstructed from computed tomography (CT) scans of 18 pig femurs, each imaged at two time points: first at 3 months of age and again at 6 months. The pre-intervention bone model was scaled and aligned to the post-intervention model. The scaled pre-intervention bone was segmented into two sections (proximal and distal), and each section was independently aligned to its corresponding post-intervention segment. Rotational changes were quantified by evaluating orientation differences between the proximal and distal femur sections before and after registration. To assess the consistency of the 3D method, segmentation and analysis were repeated. Results were compared to the 2D Lee method, which was also performed twice within a two-week interval by an expert observer.

The mean change in femoral version was 2.86 ± 1.68 degrees using the 3D method, compared to 1.43 ± 2.70 degrees with the 2D method. The 3D method demonstrated greater measurement consistency across trials (Figure 1). Intrarater reliability, assessed via the intraclass correlation coefficient (ICC), was 0.76 (95% CI: 0.47–0.90) for the 2D method and 0.97 (95% CI: 0.95–0.98) for the 3D method. A moderate correlation was noted between the 2D and 3D methods (Figure 2. A; $r = 0.52$, $p = 0.02$), with agreement further illustrated using a Bland-Altman plot (Figure 2.B).

Conclusion: The 3D method appears to be more reliable for measuring rotational changes compared to the 2D method. This approach offers significant value in identifying rotational changes in experimental studies (e.g., guided growth procedures), evaluating intervention effects, and detecting unintended changes across all three planes.

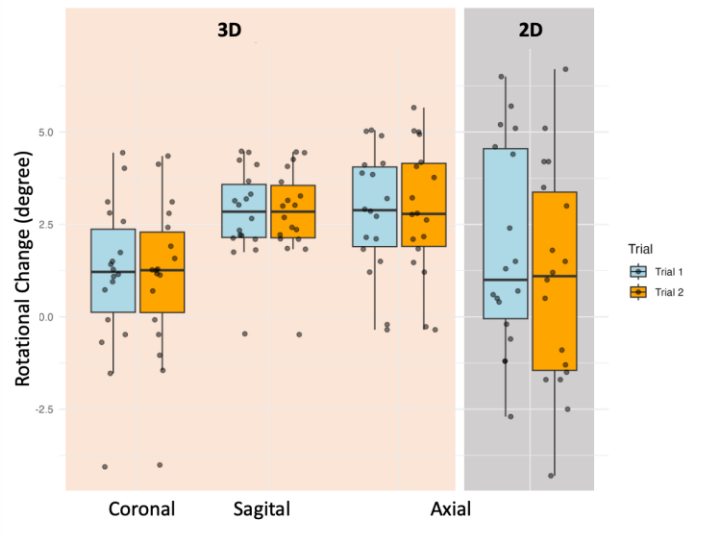


Figure 1. Box plots comparing 3D and 2D measurements of rotational changes across planes (all planes for 3D and axial plane for 2D) and two trials.

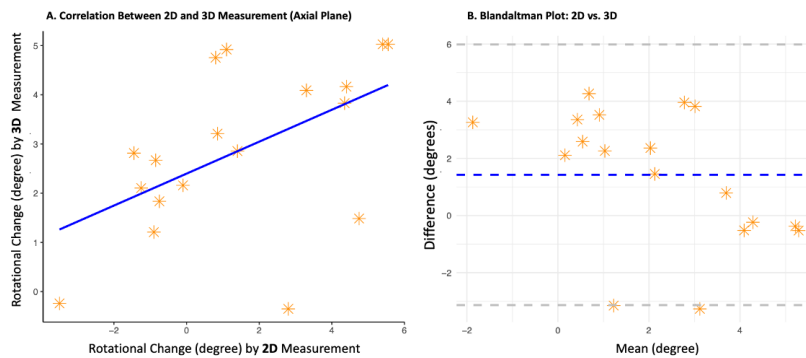


Figure 2. (A) Correlation plot demonstrating the relationship between the two methods for axial plane measurements. (B) Bland-Altman plot depicting the level of agreement between the 2D and 3D methods. The average values from Trial 1 and Trial 2 were used.

Po2_1.08

3D-Planned Corrective Osteotomy for the Treatment of Distal Radioulnar Joint Instability in Diaphyseal Forearm Malunions.

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Abstract

Forearm fractures are common in children. A potential complication is symptomatic malunion, which typically results in limited pronation and/or supination. In rare cases, rotational movement is preserved, but the malunion causes instability of the distal radioulnar joint (DRUJ) and ulnar-sided wrist pain. Soft tissue procedures are often employed to address this instability. This study evaluates the effect of a 3D-planned corrective osteotomy using patient-specific drill and cutting guides to improve DRUJ stability and reduce ulnar wrist pain.

In this retrospective cohort study, patients with a diaphyseal forearm malunion sustained during childhood, presenting with DRUJ instability but no evident limitation in forearm rotation, were included. All patients underwent a corrective osteotomy of the radius and/or ulna using 3D-printed patient-specific drill and cutting guides. The primary outcome was pain, measured by the Numeric Rating Scale (NRS) at rest and under load, six months postoperatively. Secondary outcome was subjective function, assessed using the Patient-Rated Wrist/Hand Evaluation (PRWHE) score.

Ten patients were included (mean age: 19 years; 50% male). Postoperatively, all patients demonstrated a stable DRUJ without the need for an additional soft tissue procedure. NRS pain under load decreased significantly from 5.1 to 2.4 ($p = 0.02$). Pain at rest showed no significant change. The PRWHE score improved significantly from 53 to 23 ($p = 0.04$).

A 3D-planned corrective osteotomy without adjunctive soft tissue procedures results in significant improvements in both pain and function in patients with DRUJ instability secondary to diaphyseal forearm malunion.

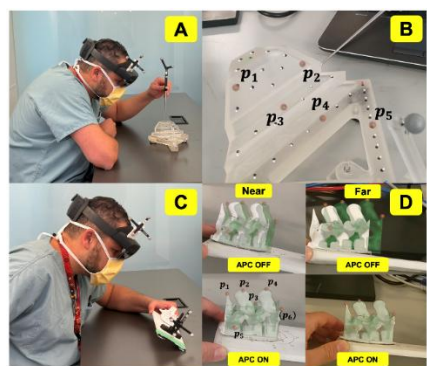


Figure 1

Experimental validation: Three right-hand-dominant orthopaedic residents (mean age = 30 ± 2 y) evaluated APC on both the calibration block and a 3-D-printed thoracolumbar spine phantom. Figure 1 shows representative scenes with APC switched OFF and ON. Projection accuracy was measured off-line by segmenting 6mm reference spheres at 6 landmarks and converting pixel-space offsets to millimetres. Table 1 summarises the mean residual error after APC: 2.12 ± 1.10 mm (near) and 2.48 ± 1.50 mm (far) on the ASTM block; 2.18 ± 1.40 mm (near) versus 2.36 ± 1.40 mm (far) on the spine—well below the 3 mm threshold considered clinically acceptable for pedicle-screw placement. Two-one-sided t-testing confirmed statistical equivalence between near- and far-field errors within a 0.9 mm margin ($p < 0.05$).

TABLE I. Mean projection errors

	Near Mean $\pm$ STD (mm)	Far Mean $\pm$ STD (mm)
ASTM	2.123 $\pm$ 1.097	2.478 $\pm$ 1.500
Spine	2.176 $\pm$ 1.396	2.356 $\pm$ 1.401
Combined	2.146 $\pm$ 1.236	2.426 $\pm$ 1.459

Implications: Compared with recent point-cloud (7.9 mm) and marker-based (≈ 2 mm) registration techniques, APC achieves superior or comparable accuracy while avoiding extra hardware, cabling and workflow disruption. By restoring far-field-level precision during close-range tasks, APC could extend OST-HMD usage from pre-operative visualisation to high-precision intra-operative drilling, burring and implant alignment in confined spinal corridors. Future multi-surgeon studies will explore inter-user variability, refine dynamic head-motion modelling, and integrate surface-based registration to generalise across cranial and pelvic morphologies.

Po2_1.09

User-specific Augmented Reality Rendering Calibration for Eliminating Inter-Subject Variability

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Abstract

Overview: Active parallax-effect compensation (APC) for surgical augmented reality (AR) aims to nullify the optical mis-registration that plagues optical see-through head-mounted displays (OST-HMDs) when surgeons lean toward the operative site. We introduce a distance-aware APC pipeline implemented on Microsoft HoloLens 2, tracked at 60 Hz by an NDI Vega XT infrared (IR) navigation system. After a single sessions of user-specific calibration, the system corrects hologram positions in real-time without external markers or hardware.

Calibration workflow: Two canonical working distances are sampled: “near” (≈ 350 mm) and “far” (≈ 800 mm). Using an ASTM F2554-22 reference block, the operator first probes six physical landmarks with an IR-tracked stylus, then acquires the corresponding virtual points rendered by the HMD. The resulting “near” and “far” 4×4 deviation matrices capture cumulative optical, rendering and eye-box errors. During surgery, the matrices are blended with a smooth hyperbolic-tangent model driven by surgeon-to-target distance, generating an adaptive correction for every vertex of the computer-generated anatomy on the OST-HMD.

Po2_2.02

Development and evaluation of silk fibroin methacrylate (SilMA)-based biomaterials for human hip acetabular labrum regeneration

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Abstract

The acetabular labrum is a fibrocartilaginous ring of tissue which surrounds the circumference of hip acetabulum, anchoring to the bone and providing joint stability. Labrum is mainly composed of fibrochondrocytes and oriented fibers of collagen type I, allowing resistance to shear stress and guaranteeing sealing properties to bone. Nonetheless, femoral-acetabular impingement can impair these characteristics by inducing labral tissue degeneration leading to hip pain, a decline in quality of life, and, ultimately, to degenerative osteoarthritis. The development of a biomaterial to create a graft for labral regeneration, to reproduce a suitable microenvironment for cell colonization with mechanical properties of native labral tissue, is of great interest.

To this aim, a keratin-silk fibroin methacrylate (keratin-SilMA) hydrogel was developed considering two different concentrations of keratin (12.5 - 25 % ww) and compared to SilMA alone. For the preparation of keratin-SilMA solutions, degummed silk fibroin was used to prepare SilMA and insoluble keratin powder from wool was dispersed into the SilMA solution, to generate keratin-SilMA hydrogels. Firstly, the ultraviolet (UV) exposure time was optimized to carry out the crosslinking process and the swelling ratio was determined to evaluate the water absorption of the hydrogel which is fundamental for the circulation of nutrients inside the material. Successively, the biocompatibility with a human chondrocyte cell line (C28/I2) was evaluated by mixing cells with biomaterial suspensions. Cell behavior in terms of apoptosis, stress, and morphology was evaluated by lactate dehydrogenase (LDH) detection in culture supernatants, histology and Transmission Electron Microscopy analyses.

The data collected showed that the keratin-SilMA hydrogels can be produced successfully with a crosslinking time of 10 minutes for all the ratios of keratin-SilMA hydrogels, without affecting cell viability. Concerning the swelling ratio, a significant difference during the first three days of culture was observed, confirming the ability of the biomaterial to properly swell, allowing the circulation of fluids in and out from the material. This is an important requirement for guaranteeing cell survival inside the construct. Moreover, biocompatibility evaluation showed that all the ratios of keratin-SilMA hydrogels allow the maintenance of cell viability until 21 days of culture, confirming the absence of cytotoxic activity.

Keratin-SilMA hydrogels were successfully created, and the crosslinking process using UV was optimized. The hydrogels showed successful fluid flow and biocompatibility with human chondrocyte cell line. Further investigations are ongoing to evaluate the production of extracellular matrix by chondrocytes when embedded in the hydrogels.

Po2_2.03

Electrochemical Behavior of Ti-Nb-Zr-Si Alloy: Potentiodynamic Study in Normal and Inflammatory Conditions

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Abstract

Metallic orthopaedic implants must exhibit sufficient corrosion resistance in the host. Inflammatory conditions impose a challenge of management of accelerated corrosion [1]. In this work long-term electrochemical behavior of novel Ti-Nb-Zr-Si (TNZS) alloy is analyzed. TNZS alloy samples were studied in normal (control) media based on phosphate-buffered saline (PBS). Inflammatory conditions were simulated with added H₂O₂ and HCl, and severe inflammatory with H₂O₂, HCl, bovine serum albumin (BSA), and calcium lactate hydrate (CLH) [1]. Potentiodynamic polarization tests were performed at 24, 48, 72, and 96h to evaluate corrosion current density (icorr).

Figure 1 shows the data for different media as E_{corr} (a) and icorr (b) vs. immersion time. The icorr behavior differed significantly across the environments. In normal conditions, a clear reduction in icorr was observed as immersion progressed from 24 to 96h, reflecting progressive passivation of the alloy. This is consistent with the stabilization of the oxide layer in the relatively benign environment. In the inflammatory medium, icorr exhibited an initially high value at 24h due to the oxidative stress induced by H₂O₂ but over time a reduction was observed, suggesting a gradual mitigation of oxidative effects as H₂O₂ being decomposed and the alloy passive layer recovers. The reduction rate was slower compared to the normal medium, highlighting the persistent challenges posed by the reactive species [2].

The severe inflammatory condition exhibited the most aggressive corrosion profile, followed by a notable reduction by 96h, indicating that the combined effects of H₂O₂, BSA, and CLH led to accelerated corrosion and delayed passivation. Compared to the inflammatory medium, the reduction in icorr was less pronounced, emphasizing the synergistic role of BSA and CLH in promoting localized attack. Interestingly, the decreasing icorr trend suggests that the alloy's microstructure and the presence of silicide phases offered enhanced corrosion resistance, preventing uncontrolled degradation [1]. The relative rate of reduction in icorr was comparable between inflammatory and severe inflammatory conditions after 48h, suggesting that the long-term behavior of the TNZS alloy is influenced by its intrinsic ability to stabilize the passive film under oxidative and biomolecular attack.

Prolonged immersion tests confirm the TNZS alloy's corrosion resistance, with decreasing icorr, and its stability under severe inflammatory conditions underscores its potential for long-term orthopedic applications.

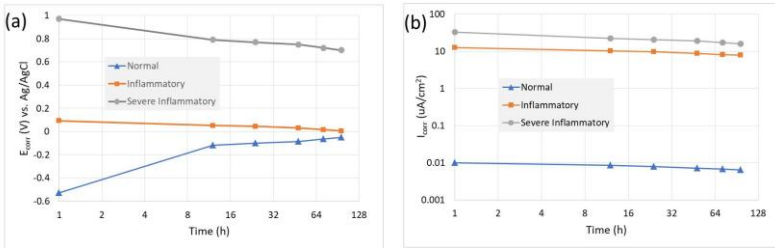


Fig.1: (a) E_{corr} and (b) i_{corr} values extracted from Tafel extrapolation.

Acknowledgement: Grateful for financial support from the

EU “Premurosa” (GA 860462) project.

[1] Bordbar-Khiabani A, Gasik M. *Scientific Reports*;13(1):2312.

Po2_2.04

Elucidating the Molecular Mode of Action of the Osteogenic P-15 Peptide: A Systematic Literature Review

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1Maastricht University, Maastricht, Netherlands. 2Maastricht University Medical Center, Maastricht, Netherlands. 3CeraPedics, Denver, USA. 4Eindhoven University of Technology, Eindhoven, Netherlands

Abstract

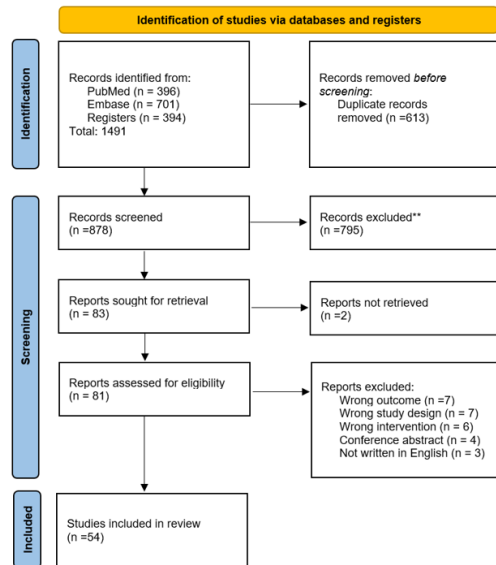
Bone is a dynamic tissue with the ability to regenerate and remodel. However, its regenerative capacity reduces over time, as the number of stem cells and growth factors diminishes with age. While various bone graft materials and substitutes have been developed, including autologous bone grafts, bone morphogenic proteins (BMPs) and ceramic materials, each present with significant limitations. Bioactive synthetic peptides have been developed to enhance bone regeneration and have been considered as an alternative to current treatment techniques. The synthetic P-15 peptide is a 15-amino acid sequence derived from human collagen type I. This peptide has demonstrated the ability to attract, attach and activate osteoprogenitor cells, clinically resulting in mature bone grafts. However, a comprehensive evaluation of the molecular evidence underlying these processes has not yet been fully elucidated. This systematic review aims to provide a comprehensive overview and critically assess the supporting evidence for the molecular mechanisms driving the attraction, attachment and activation phase of the P-15 peptide in bone regeneration.

This review, pre-registered in PROSPERO (CRD42024395851), adhered to the PRISMA guidelines. In vitro research relating to the molecular mode of action and the biological impact of P-15 were included, as well as in vivo animal studies. Exclusion criteria encompassed studies before 1990, human clinical research, P-15 combined with other synthetic bone grafts or blood products, and literature reviews. Searches were conducted in Medline (Pubmed), Web of Science (WoS) and Embase (OVID) databases. Two researchers independently performed screening and data extraction using Rayyan and Covidence software. Risk of bias was assessed using the QUIN Tool for in vitro studies and the SYRCLE's risk of bias tool for animal studies. Studies were categorized based on the three molecular mechanism phases of P-15's mode of action.

After duplicate removal, 878 papers were screened. Ultimately, 54 papers met the inclusion criteria and were included for data extraction. Qian and Bhatnagar (1996) demonstrated increased cell attachment with increasing P-15 concentration on ABM particles, accompanied by enhanced ALP production. Yang et al. (2004) confirmed P-15's potential by

providing evidence for improved adhesion of osteoprogenitor cells to ABM/P-15 scaffolds. Liu et al. (2012) demonstrated that P-15 attachment to titanium surfaces enhanced cell attachment, osteogenic gene expression, and differentiation, thereby promoting bone formation and osseointegration.

This systematic literature review substantiated the evidence of the three phases of the molecular mode of action of the P-15 peptide and highlighted the promising role of P-15 in bone regeneration.



PRISMA 2020 flow diagram of the study selection process for the systematic review of P-15's molecular mode of action.

Po2_2.06

Synergistic Influence of Mesoporous Bioactive Glass Particle Size and Concentration on the Osteogenic Potential of Calcium Phosphate Cements

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Abstract

Integrating mesoporous bioactive glass (MBG) into calcium phosphate cements (CPCs) presents a promising strategy to overcome the clinical limitations of bone cements, including limited mechanical strength and slow degradation rates. This study uniquely explores the dual impact of MBG particle size (<20 μm , <38 μm) and content (7 wt. % and 9 wt.%) on CPCs, focusing on setting behavior, mechanical integrity, ion release, and osteogenic differentiation using human mesenchymal stem cells (hMSCs). MBG particles smaller than 20 μm significantly reduced setting time while improving compressive strength (~10.4 MPa) and enhancing cell viability (>92 %), particularly at 9 wt. % concentration. Notably, the smaller MBG particles promoted a more homogeneous microstructure, resulting in optimized ion release (Ca: 80.4 $\mu\text{g/mL}$, P: 705.4 $\mu\text{g/mL}$, Si: 130.4 $\mu\text{g/mL}$) and enhanced early-stage mineralization. Gene expression analysis revealed upregulation of early osteogenic markers (ALP, COL1, and RUNX2) with increased MBG content, while late-stage differentiation markers (OC) remained underexpressed, emphasizing the need for extended differentiation periods to fully capture osteogenic capacity. IBSP staining further confirmed enhanced mineralization in MBG-modified composites, particularly in 9 wt. % MBG content formulations. Although the differentiation effect was not statistically significant, these findings highlight MBG's potential for ion-mediated bioactivity enhancement, consistent with previously reported osteoinductive properties *in vivo*, as demonstrated in the literature. This study emphasizes the importance of fine-tuning both MBG size and concentration to optimize CPC performance for bone regeneration applications.

Po2_2.07

“Biodigital Twins”: optimizing biomaterials for orthopaedic implants

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Abstract

Despite the success of implants, there is still a risk of infections - complications drastically compromising patients' quality of life, causing pain & immobility. The formation of stable and virulent biofilm by causative pathogens affected by different factors is central to the occurrence and the recalcitrance of these infections.

Any implant has a distinct set of features (surface quality, porosity, topology, etc.) linked with basic properties (wettability, surface energy, charge, etc.) and interacts with liquids, proteins, bacteria and cells in a set complex interactions not exactly known. However it is evident that we want such implant surface, which would have the least infection/inflammation risks and the best osteogenesis in vivo. The challenge is that we do not know which surface it would be like, because of huge varieties of input factors and variables cross-correlated in unknown ways. Technically, this becomes a task for a multi-objective design and optimization (MODAO) and can be approached with machine-learning algorithms (MLA).

In this work we demonstrate analysis of the set of many parameters with MODAO - different surfaces of titanium alloy (roughness, porosity, topology, pore size, wetting angle), biofilm formation factor, cells adhesion/proliferation (<27 days), gene expression, inflammatory potential in vitro, data from in vivo rabbit model - analysed with modern methods to select the "best" choice from the list using multi-criteria decision making (MCDM). Here we selected only those variables which could be directly engineered and controlled.

The results indicate that even with scarce scattered data it is possible to reveal links between variables and estimate most important factors. Porosity, wetting and roughness are major parameters affecting performance of the biomaterial, and biofilm formation is negatively correlated to cells proliferation and growth, but less with gene expression markers. Analysis has revealed several important correlations for inhibition of biofilm and improvement of cell reactions and has shown potentially interested directions for further developments. Application of MODAO MLA to larger datasets could lead to the better selection of surface/coating of the implant and its surface engineering methods to reduce risks and improve the performance.

Acknowledgement: support from the EU framework program for projects MEDDELCOAT, PANBIORA, PREMUROSA and EXCELLMATER.

Po2_2.10

Physicochemical characterization and in vitro-evaluation of Bi-doped dendritic mesoporous bioactive glass nanoparticles for bone void-filling applications

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Abstract

Bismuth-doped dendritic mesoporous bioactive glass nanoparticles (Bi-DMBGNs) with potential for bone void-filling applications were prepared and characterized. Cell viability, bioactivity, radiopacity, and antibacterial activity were evaluated to assess their suitability in bone tissue engineering. Four different compositions of mesoporous bioactive glass nanoparticles in the SiO₂-CaO system were synthesised using the sol-gel method, while bismuth was incorporated through posterior impregnation followed by calcination. To incorporate Bi, dried bioactive glass nanoparticles were dispersed in ethanol solution of bismuth (III) nitrate pentahydrate at two concentrations, 0.01 M and 0.05 M, with a nanoparticle concentration of 16 mg/mL. The impregnation process was conducted under two temperature conditions: room temperature and above the melting point of bismuth (III) nitrate pentahydrate to evaluate the effects of both concentration and temperature. This approach aims to optimise the performance of the composite by utilizing specific and beneficial properties of bismuth, including radiopacity and potential therapeutic effects, while maintaining the structural characteristics of mesoporous bioactive glass. Various techniques were used to characterise the Bi-DMBGNs. Scanning electron microscopy (SEM) was used to analyse the morphology, size, and surface structure, confirming the dendritic mesoporous character of the nanoparticles. Optical emission spectrometry with inductively coupled plasma (ICP-OES) was used to determine the elemental compositions of Bi-DMBGNs with progressively increasing addition of Bi₂O₃. The Brunauer-Emmett-Teller (BET) adsorption model was employed to assess the specific surface area and pore volume. Fourier Transform Infrared Spectroscopy (FTIR) provided insights into the molecular structure. The zeta potential measurements were used to evaluate surface charge, which affects cellular interactions and stability of the nanoparticles in the biological media. The radiopacity of DMBGNs with bismuth doping and its potential to improve imaging contrast in radiographic studies was also determined using an aluminium step-wedge test. The in-vitro bioactivity of the Bi-DMBGNs was tested through apatite formation on the particles surface after immersion in simulated body fluid (SBF). The samples were also tested for antibacterial activity while assessing cell viability using a human osteosarcoma MG-63 cell line. The tests were conducted to evaluate the potential of Bi-DMBGNs as a material for treating bone defects and promoting bone regeneration (e.g., drug delivery systems, bone void fillers, and cement, among others), offering enhanced radiopacity, bioactivity, and antibacterial properties.

Po2_2.12

Mimicking physiology: Perfusion bioreactor characterisation of the polyphenol-functionalized hydrogels for controlled drug delivery

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Abstract

Understanding the characteristics and release behavior of therapeutic compounds from complex controlled-release systems under physiological conditions remains a challenge in drug development. Biomimetic bioreactors provide a more accurate and reliable platform by replicating the dynamic physiological conditions of targeted tissues and applications.

In this study, we aimed to develop a localized anticancer drug delivery system based on methacrylate chitosan hydrogels (MCH) functionalized with green tea polyphenols (GTP) or tannic acid (TA). We hypothesized that MCH prepared via UV-curing and subsequently functionalized with GTP or TA, would exhibit enhanced mechanical stability and enable the effective, sustained release of bioactive compounds.

The primary objective of this study was to investigate the release behavior of polyphenols from MCH over 7 days under *in vivo*-like conditions, using a continuous flow of culture medium in a perfusion bioreactor at a superficial medium velocity of 200 $\mu\text{m/s}$ which corresponds to capillary blood flow. Additionally, we aimed to evaluate the MCH mechanical properties and release kinetics to better understand their potential as controlled drug delivery carriers.

MCH were synthesized by UV crosslinking of a 1.5% wt. methacrylate chitosan solution with 2% wt. photoinitiator, followed by air drying, and subsequently functionalized with GTP or TA by immersing the dried hydrogels in 5 mg/cm^3 solutions for 3 h. Mechanical characterization of both pure and functionalized hydrogels (before and after polyphenol release) was conducted in a bioreactor under dynamic compression at 10% strain. To determine the apparent diffusion coefficient and net fluid velocity, the release kinetics of GTP and TA were analysed in PBS under both static and perfusion conditions.

Additionally, the prolonged polyphenol release was monitored over 7 days, and the anticancer efficacy of the released compounds was assessed using murine osteosarcoma K7M2-wt cells.

The hydrogels maintained mechanical stability after 7 days in culture medium. Functionalization with TA or GTP significantly improved the mechanical properties by approximately 1.5-fold compared to initial hydrogels. After 7 days under static conditions,

mechanical properties nearly returned to the baseline, while hydrogels subjected to perfusion in the bioreactor exhibited an almost 4-fold reduction in mechanical strength, yet remained mechanically stable (dynamic compression modulus ~145 kPa). Released polyphenols demonstrated moderate anticancer activity against murine osteosarcoma K7M2-wt cells. These findings highlight the potential of polyphenol-functionalized methacrylate chitosan hydrogels as promising candidates for localized drug delivery in cancer therapy and underscore the advantages of biomimetic bioreactors in bridging the *in vitro–in vivo* gap.

This research was supported by the Science Fund of the Republic of Serbia, grant no. 7503, Biomimetic Tumor Engineering to Enhance Drug Discovery - BioengineeredTumor.

Po2_2.14

Next-Generation Biomaterials: Mesoporous Bioactive Glasses Enhance the Performance of Calcium Phosphate Bone Cements

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Abstract

Calcium phosphate cements (CPCs) are widely recognized for their osteoconductive properties and clinical potential in bone regeneration, but are limited by poor mechanical strength, prolonged setting times and slow degradation rates. On the other hand, mesoporous bioactive glasses (MBGs) offer a promising approach to enhance the compressive strength of CPCs, providing a high surface area, pore structure, and pore volume, which are highly advantageous for tissue regeneration. A combination of CPCs and MBGs can be utilized in titanium orthopaedic implants, where titanium provides exceptional mechanical support, and the CPC-MBG composites contribute biocompatibility and bioactivity. By applying CPC-MBG composites as coatings, these implants can promote bone integration and regeneration, effectively addressing both the mechanical and biological requirements of orthopaedic applications while overcoming the inherent limitations of CPCs. This study focuses on integrating MBG particles into CPC formulations to assess their influence on setting behavior, microstructure, mechanical properties, and in vitro cellular response. Sol-gel synthesized MBG particles were added to CPCs at different concentrations (0–15 wt.%) to evaluate their effects on cement properties. MBG addition significantly influenced the setting characteristics and mechanical strength. Low concentrations (≤ 9 wt.%) of MBG enhanced cement injectability, cohesion, and compressive strength, achieving values up to ~ 10.4 MPa, suitable for non load-bearing applications. Furthermore, the incorporation of MBG promoted ion release from CPC-MBG composites, facilitating apatite formation. Finally, the degradation rates and bioactivity of CPC-MBGs were optimized, as demonstrated by favourable cytocompatibility with over 92% cell viability compared to controls. Real-time polymerase chain reaction (RT-PCR) confirmed the successful expression of human gene markers, further validating the materials' ability to support osteogenic differentiation.

Acknowledgment: This work has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE – Baltic Biomaterials Centre of Excellence).

Po2_2.18

Templated Synthesis of Hollow RuO₂ Nanospheres for Alleviating Metal Wear Particle-Induced Osteoclast Activation and Bone Loss

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Abstract

Wear particulate debris-induced osteoclast over-activation is a major causative factor in periprosthetic osteolysis and aseptic loosening, resulting in the non-infectious failure of total joint arthroplasty. To mitigate such pathological bone loss, various therapeutic targets have been identified. Oxidative stress is one of the main contributing factors in the promotion of osteoclastogenesis. Herein, using a template-assisted approach, we synthesized hollow ruthenium oxide (RuO₂) nanospheres as an effective antioxidant for the treatment of CoCrMo alloy particles-induced osteolysis. Hollow RuO₂ significantly suppressed receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclastogenesis, bone resorption, and the expression of specific markers (including NFATc1, c-Fos, MMP9, CTSK, and DC-STAMP) of bone marrow-derived macrophages via inhibition of the nuclear factor kappa-B (NF-κB) and tumor necrosis factor (TNF)-α pathways. In mouse calvaria, hollow RuO₂ nanozymes demonstrated an appreciable attenuation in osteoclast differentiation, bone loss, and soft tissue malformations induced by CoCrMo alloy particles. We envision that the antioxidant nanozymes may be further applied as a therapeutic entity in the prevention and treatment of other inflammatory and oxidative stress-related ailments.

Po2_2.20

Dual-targeted Therapy Based On The Macrophage Niche In Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is characterized by inflammatory infiltration and bone destruction, primarily due to macrophage niche disturbances. We identified a key process where complement overactivation disrupts the barrier function of VSIg4+ lining macrophages in RA, leading to increased inflammatory infiltration and osteoclastogenesis, resulting in extensive bone resorption. However, complement antagonists are often limited by their super-physiologic dosages and minimal impact on bone resorption.

We analyzed single-cell sequencing data from synovial CD45+CD11b+Ly6G- monocyte-derived macrophages (dataset GSE134420) to explore the relationship between complement activity and macrophage imprinting in RA. To combat excessive complement activation, we developed a novel recombinant fusion protein, CRlg-CD59, and tested its efficacy in a rat antigen-induced arthritis (AIA) model. Despite dose dependencies and limited effects on bone resorption, we further developed a dual-targeted therapeutic nano-platform using a metal-organic framework (MOF), which allowed bone-targeted delivery and pH-responsive release of CRlg-CD59. The efficacy of this platform was confirmed through Micro-CT, histological staining, and immunofluorescence analyses on treated AIA rats.

GO and KEGG analyses reinforced the central role of the complement cascade in RA. The recombinant CRlg-CD59 protein was efficiently expressed and exhibited significant anti-complement activity. Although high-dose CRlg-CD59 reduced inflammatory markers like IL-6 and TNF- α in the AIA model, it did not significantly alleviate bone destruction. The dual-targeted nanoplateform, ZIF8@CRlg-CD59@HA@ZA, demonstrated effective pH-dependent release, complement inhibition, bone targeting, and therapeutic activity in RA. Animal studies and single-cell sequencing confirmed that this therapy effectively reduced complement and osteoclast activity, adjusted macrophage populations, and improved the macrophage niche.

Various strategies using complement inhibitors have shown promise in animal models of RA. However, some, like the C5aR inhibitor PMX53 and anti-IL-6 receptor antibody tocilizumab, were ineffective in clinical settings. These findings highlight the need for further research into complement as a therapeutic target in RA. Our study underscores the limitations of single drug components and suggests that nanoplateforms can provide more effective and comprehensive treatment options through their ability to deliver drugs

specifically and adjust to the pathological complexities of diseases like RA. This opens new avenues for the design and development of future RA treatment strategies.

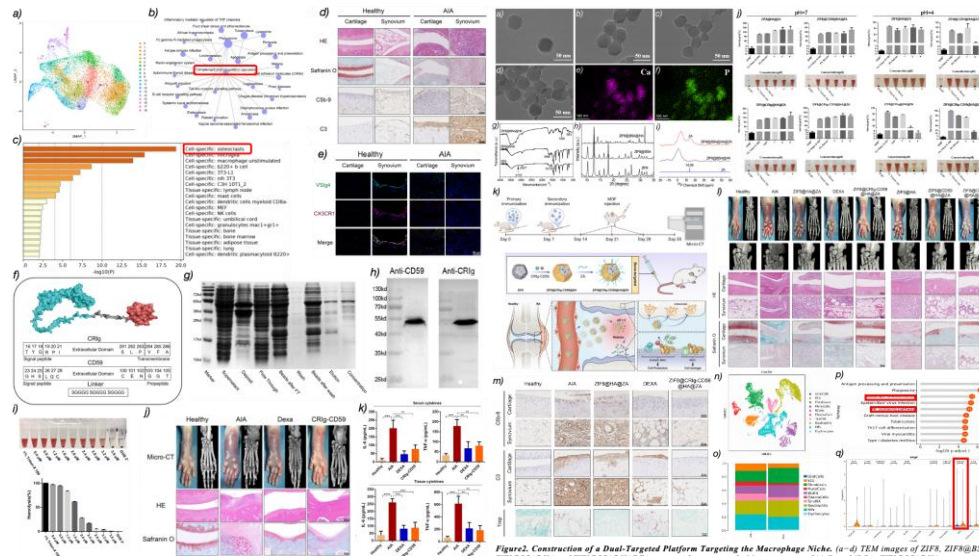


Figure1. Identifying the Characteristics of the Macrophage Niche in Rheumatoid Arthritis. (a) UMAP analysis of synovial CD45⁺ CD11b⁺ Ly6G⁻ macrophages delineates the distribution of 18 subpopulations. (b) Pathway enrichment of DEGs revealed by KEGG. (c) Cell-specific enrichment of DEGs was determined in Ingenuity. (d) HE and safranin O joint green staining, DIC for CS-9 and CS. (e) Immunofluorescence staining for Y324 and CD59 of SD rat joints. (f) Schematic diagram of CRlg-CD59 fusion protein design and mock protein structure. (g) SDS-PAGE image of the preparation process of CRlg-CD59 protein. (h) Western blot detection of CRlg- and CD59-specific antibodies of CRlg-CD59 recombinant protein. (i) Complement hemolysis inhibitory activity of fusion protein CRlg-CD59. (j) Micro-CT, HE staining, and safranin O joint green staining of joints in AIA model rats treated with CRlg-CD59 and deamethasone for 21 days. (k) Serum and tissue IL-6 and TNF- α protein levels in AIA rat model. Data are presented as mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. All results are representative data generated from at least three independent experiments. The one-way ANOVA with the Tukey's multiple comparison test was used for statistical analysis.

Figure2. Construction of a Dual-Targeted Platform Targeting the Macrophage Niche. (a-d) TEM images of ZIF8, ZIF8@BSA, ZIF8@BSA@HA, and ZIF8@BSA@HA@ZA nanoparticles, respectively. Mapping images of (e) Ca and (f) P in ZIF8@BSA@HA nanoparticles. (g) FTIR spectra of ZIF8, ZIF8@BSA and ZIF8@BSA@HA nanoparticles. (h) XRD patterns of ZIF8, ZIF8@BSA, and ZIF8@BSA@HA nanoparticles. (i) 31P NMR spectra of ZA (in D2O solution), ZIF8@BSA@HA (solid), and ZIF8@BSA@HA@ZA nanoparticles (solid). (j) Complement hemolytic activity assay of ZIF8@HA@ZA, ZIF8@CD59@HA@ZA, ZIF8@CRlg@HA@ZA, and ZIF8@CRlg-CD59@HA@ZA at pH 7 and 4. (k) Mechanism diagram of ZIF8@CRlg-CD59@HA@ZA treatment of AIA. (l) Representative histology micro-CT images of an AIA rat, each with a magnified image of the ankle joint. Cartilage and synovium after joint tissue samples were processed with hematoxylin and eosin (HE) and safranin O joint green staining. (m) Cartilage and synovial tissues were analyzed for CS-9 by immunohistochemistry, immunohistochemical analysis of CS and tartrate acid phosphatase staining. (n) UMAP dimensionality reduction plot for unsupervised clustering of cell subtypes. (o) Percentage distribution of overall subclusters. (p) KEGG enrichment Lollipop of overall downregulated differentially expressed genes. (q) Violin plot of Y324 expression in cell subpopulations after treatment with the dual-targeted platform.

Po2_2.37

Breathing Micelles For Combinatorial Treatment Of Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis progressively impairs joint function and ultimately leads to disability, significantly reducing the quality of life and posing a serious public health problem. Higher organisms, including animals and plants, generate energy through respiration to maintain normal physiological functions. In the typical process of aerobic respiration, oxygen (O₂) is inhaled and carbon dioxide (CO₂) is exhaled for gas exchange. In addition to O₂ and CO₂, various gases such as carbon monoxide (CO) and nitric oxide (NO) are produced by organisms. NO is notably overproduced in RA, and its selective scavenging has shown promise in treatment strategies. Despite their reputations as pollutants, NO and CO play vital roles in regulating physiological and pathological processes, including inflammation. High NO levels correlate with inflammatory diseases, while CO has anti-inflammatory effects. To harness these properties, we developed a biomimetic approach using respiratory micelles that simulate gas exchange, combining pro-inflammatory NO and anti-inflammatory CO for potential RA therapy.

We designed a Breathing Micelles (BM) platform that "inhales" NO and "exhales" CO. These nanoparticles were functionalized with ortho-phenylenediamine (oPDA) for NO reaction and 3-hydroxyflavone (3-HF) derivatives for CO release, facilitated by a benzotriazole (BTA) structure and activated by visible light. BMs were characterized through various spectroscopic and fluid dynamics techniques, and their functionalities were tested in vitro for biocompatibility, NO scavenging, CO release, and anti-inflammatory activity. Their effectiveness was further evaluated in a rat model of adjuvant-induced arthritis (AIA).

The biomimetic micelles (BM) demonstrated a stable morphology with an average diameter of 32 nm, maintaining stability under continuous nitric oxide (NO) treatment and light exposure. UV-Vis spectroscopy and NMR confirmed the formation of benzotriazole (BTA) derivatives in the micelles, which facilitated controlled carbon monoxide (CO) release upon light activation, leading to a reduction in specific absorbance peaks. Functionally, BMs showed high biocompatibility and efficient cellular uptake by RAW264.7 cells. They effectively scavenged excessive NO and reduced inflammatory markers like IL-6 and TNF- α . In a rat model of adjuvant-induced arthritis, BMs outperformed both single-gas components

and dexamethasone, showing superior anti-inflammatory and therapeutic benefits, evidenced by improved joint morphology and reduced inflammation markers.

The novel biomimetic BM effectively mimics respiratory gas exchange, simultaneously scavenging harmful NO and releasing therapeutic CO. This dual-functionality offers enhanced anti-inflammatory effects, making BM a promising therapeutic strategy for RA, surpassing the efficacy of traditional treatments and individual gas-targeting approaches.

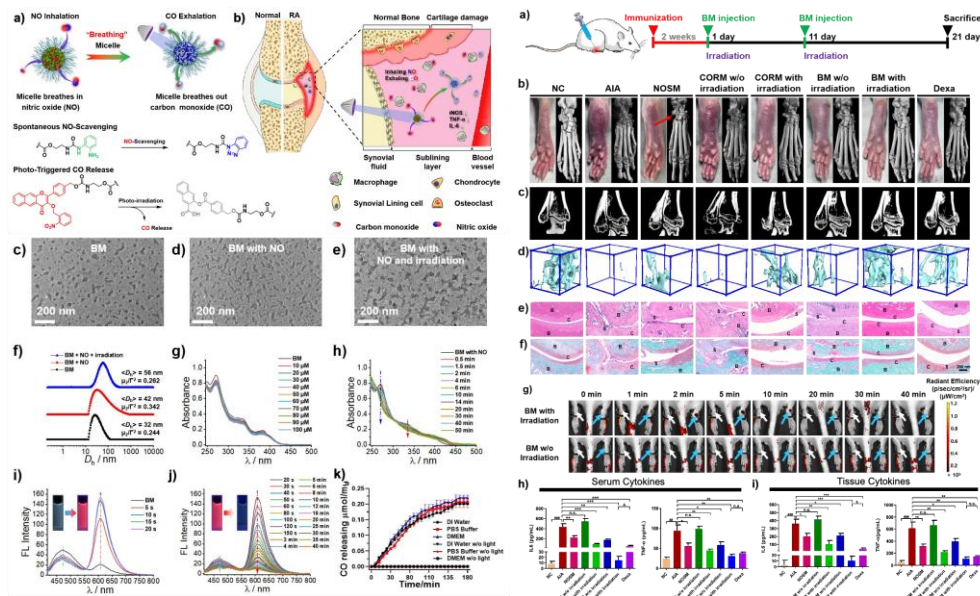


Figure 1. Synthesis and in vitro characterization of BM. (a) Schematic of the "breathing" micelle (BM) mechanism. (b) TEM images and DLS data of BM with and without NO. (c) UV-Vis spectra of BM with and without NO. (d) Fluorescence spectra of BM with and without NO. (e) CO release from BM under different conditions. (f) CO release from BM under different conditions. (g) CO release from BM under different conditions. (h) CO release from BM under different conditions. (i) CO release from BM under different conditions. (j) CO release from BM under different conditions.

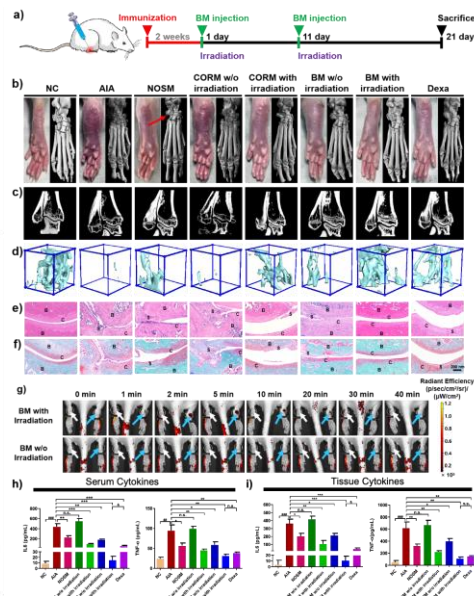


Figure 2. In vivo anti-RA activity of BM. (a) Experimental timeline for in vivo RA treatment. (b) Representative images of joint morphology. (c) Representative images of joint morphology. (d) Representative images of joint morphology. (e) Representative images of joint morphology. (f) Representative images of joint morphology. (g) Representative images of joint morphology. (h) Representative images of joint morphology. (i) Representative images of joint morphology. (j) Representative images of joint morphology.

Po2_2.23

Effect of LIPUS on the degradation behavior of magnesium alloy scaffolds for bone repair: Insights from *in vitro* and *in vivo* studies

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Abstract

Magnesium (Mg) alloys have excellent biocompatibility and biodegradability, making them promising for clinical applications. However, their rapid degradation compared to bone healing limits their effectiveness. This study aims to investigate the degradation behavior of bare Mg alloy scaffolds and their interactions with bone healing under the influence of LIPUS. ZE21C alloy was chosen as a scaffold material because of its good biocompatibility and suitable DR. *In vitro* experiments were performed in Hanks' balanced salt solution (HBSS) + BSA solution to assess the degradation of the scaffolds, while *in vivo* studies were performed by implanting the scaffolds into the femoral condyles of male SD rats. The effects of LIPUS on scaffold degradation and bone repair were systematically investigated, and the results provide insight into the potential clinical application of combining LIPUS with Mg scaffolds for the treatment of bone defects.

The main conclusions drawn from the experiment are as follows:

- LIPUS accelerated Mg scaffold degradation via mechanical stimulation, demonstrating its potential to modulate degradation and improve bone implant performance.
- Under LIPUS stimulation, the O-containing layer thickened both *in vivo* and *in vitro*, while the trends for the P- and Ca-containing layers were opposite in both conditions.
- LIPUS accelerated the conversion of trabeculae, resulting in better quality and more mature new bone at the site of the defect.

In conclusion, LIPUS combined with Mg alloy scaffolds regulates degradation, promotes bone regeneration, and highlights the role of mechanical stimulation in enhancing biodegradable scaffolds for bone repair applications.

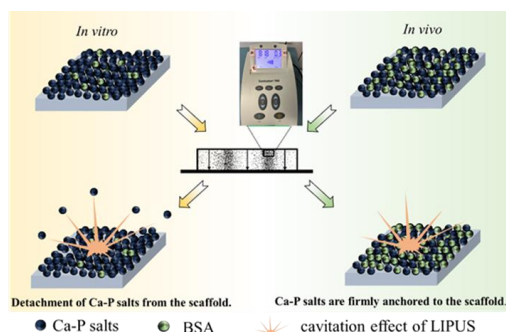


Fig. 1. Mechanism of LIPUS action.

Po2_2.24

Near-Infrared Light-Responsive Nanozymes for Reversible Modulation of ROS to Regulate Osteogenic Differentiation

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Abstract

Nanozymes have emerged as promising biocatalysts for modulating intracellular biochemical reactions, offering potential for advancing biological understanding and therapeutic innovation. However, achieving dynamic and reversible regulation of their catalytic activity remains a challenge. Here, we introduce a novel approach leveraging near-infrared (NIR) light to control nanozyme activity via a hydrophobic-hydrophilic interface switch. In the "off" state, nanozymes possess a hydrophobic surface that limits substrate access, restraining enzymatic activity. Upon NIR irradiation, the surface becomes hydrophilic, activating the catalytic function and enabling precise modulation of intracellular reactive oxygen species (ROS). By dynamically controlling ROS levels, this system effectively influences both Wnt/ β -catenin and BMP/Smad signaling pathways critical for osteogenic differentiation. The reversible nature of the system ensures that ROS levels are optimized to promote differentiation while avoiding oxidative stress and associated side effects. This strategy offers a non-invasive, spatiotemporally controlled method for bone regeneration therapies and a powerful tool for studying osteogenic processes with unprecedented precision.

Po2_2.25

Stem Cell-Laden Hydrogels With Biomimetic Microenvironment for Osteochondral and Cartilage Tissue Engineering

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Abstract

Osteochondral damage from trauma or osteoarthritis is a general joint disease that can lead to an increased social and economic burden in the modern society. The inefficiency of osteochondral defects is mainly due to the absence of suitable tissue-engineered substrates promoting tissue regeneration and replacing damaged areas. The hydrogels are becoming a promising kind of biomaterials for tissue regeneration. The biomimetic hydrogel microenvironment can be tightly controlled by modulating a number of biophysical and biochemical properties, including matrix mechanics, degradation, microstructure, cell adhesion, and intercellular interactions. In particular, advances in stem cell-laden hydrogels have offered new ideas for the cell therapy and osteochondral repair. Herein, we report the importance of stem cell-laden hydrogels on promoting the development of osteochondral regeneration, especially in the field of manipulation of biomimetic microenvironment and utilization growth factors with various delivery methods.

Acknowledgements . Project supported by the National Natural Science Foundation of China (NSFC, 82002298, 51920105006, 51973226).

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Po2_2.26

Comparative Study of Mechanical and Biological Properties of Decellularized Extracellular Matrix Hydrogels Prepared Using Two Crosslinking Strategies for Cartilage Repair

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Abstract

Decellularized extracellular matrix (dECM) hydrogels have broad applications in cartilage repair. Ensuring that dECM retains its functionality after being incorporated into hydrogels while pursuing the optimal mechanical properties of the hydrogels is critical. Hence in this work our goal was to identify an optimal method for fabricating dECM hydrogels for cartilage regeneration.

In this study, hyaluronic acid-tyramine 3% w/v (THA) was used as the hydrogel precursor and 20% dECM particles from bovine cartilage was added. Two photo-crosslinking methods were compared: **1.** Horseradish peroxidase 0.1 U/mL / eosin 0.2 mg/mL (HRP/Eo) and **2.** Sodium persulfate 5 mM / ruthenium 1 mM (SPS/Ru). Rheology test was performed to assess the viscoelastic property of hydrogels during gelation phase. Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the effect of dECM on hydrogel crosslinking. *In vitro* study was performed to evaluate the biological and biomechanical property of hydrogels seeded with 5 million/mL chondrocytes cultured for 14 days. The mechanical properties of the hydrogels were evaluated by compressive test. Histological staining, as well as DNA, GAG, and COL content analysis, were performed to compare the neocartilage generation performance of different crosslinking methods. RT-qPCR was used to evaluate cartilage regeneration-related gene expression and *in situ* fluorescence labeling were performed to assess newly synthesized GAG.

Amplitude sweep results showed that dECM did not affect the linear viscoelastic region (LVR) of the hydrogels, while SPS/Ru exhibited a lower LVR upper strain limit. Frequency sweep results indicated similar adaptability under dynamic loading for both hydrogels, with no significant impact from dECM. FTIR analysis indicated that dECM was involved in the crosslinking process, with distinct mechanisms observed between the two methods. Compressive test showed that dECM significantly increased hydrogel stiffness, and after 14 days of culture, the HRP/Eo-dECM exhibited a higher compressive modulus than the SPS/Ru-dECM (Fig. 1).

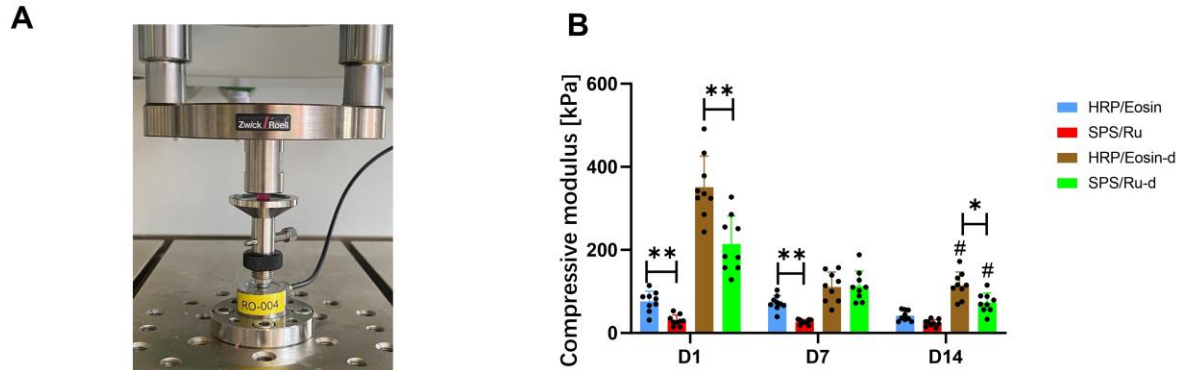


Fig. 1. Characterization of hydrogel mechanical properties by compression testing with (A) depicting used setup and (B) showing compressive modulus as a function of time of hydrogels containing cells, with dECM (abbreviated as -d) or without.

Safranin O/Fast Green staining demonstrated that all hydrogels maintained structural integrity after 14 days of culture. GAG and COL content revealed that HRP/Eo preserved more GAG and COL. Gene expression showed that chondrocytes expressed higher levels of cartilage regeneration-related genes in dECM hydrogels prepared with HRP/Eo (Fig. 2).

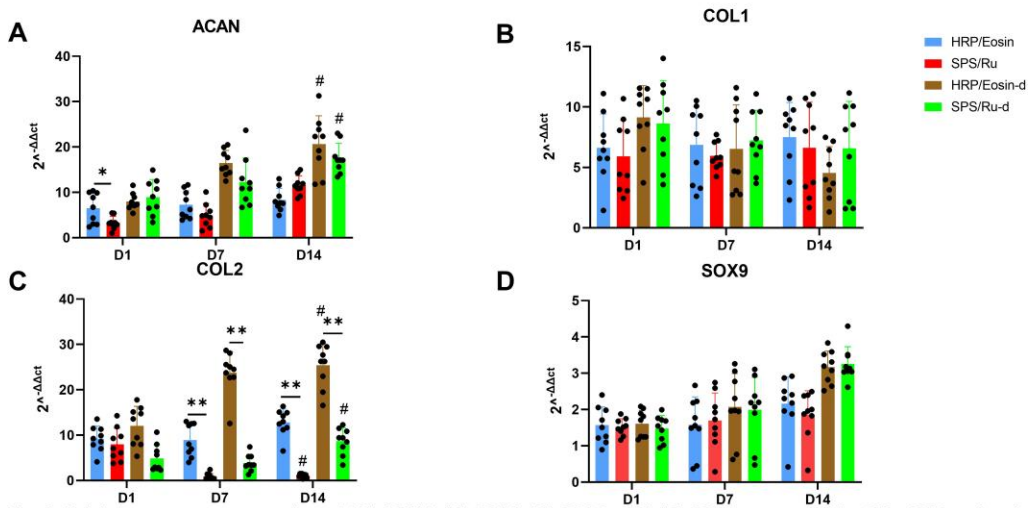


Fig. 2. Fold change gene expression of (A) ACAN, (B) COL1, (C) COL2, and (D) SOX9, measured by RT-qPCR at day 1, 7 and 14 of culture on hydrogels from both methods, either containing dECM (abbreviated as -d), or without.

In situ fluorescence labeling showed that no significant differences between the two methods.

HRP/Eo demonstrates higher stiffness and exhibits superior chondrocytes redifferentiation effect compared with SPS/Ru under tested conditions, indicating its possible use for cartilage regeneration therapy.

Po2_2.31

In-situ Sprayed platelet-derived small extracellular vesicles for the skin flap survival by reducing PANoptosis

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Abstract

Necrosis at the distal end of random skin flaps remains a significant challenge, limiting the clinical application of these flaps in plastic and reconstructive surgery. Inhibiting ischemia/reperfusion (I/R) injury and promoting the formation of neovascular networks are critical preventive strategies. Platelet-derived small extracellular vesicles (PL-sEV) are nanocarriers of growth factors that provide an alternative to clinically used platelet-rich plasma and platelet lysates, offering higher growth factor concentrations and lower immunogenicity. In this study, PANoptosis, a distinct form of inflammatory cell death, was fully characterized in a random skin flap model. Subcutaneous injection of PL-sEV improved ischemic skin flap survival by enhancing blood perfusion and reducing PANoptosis levels. In vitro, PL-sEV inhibited oxygen-glucose deprivation/reoxygenation-induced dysfunction in human umbilical vein endothelial cells. Furthermore, PL-sEV was incorporated into a thermosensitive triblock hydrogel, creating a sprayable delivery system (PLEL@PL-sEV). Mechanistic analysis through RNA sequencing indicated that the protective effects of PL-sEV against PANoptosis likely resulted from its anti-inflammatory properties, particularly via suppression of the NF- κ B signaling pathway. This novel hydrogel system demonstrated controlled release of PL-sEV and proved effective in improving skin flap transplantation outcomes.

Po2_2.32

Decellularized-disc based allograft and xenograft prosthesis for the long-term precise reconstruction of temporomandibular joint disc

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Abstract

Currently, no effective disc reconstruction treatment strategy is clinically available for temporomandibular joint (TMJ) disc-related diseases. To address this, we developed a prosthesis construct with laser-drilled decellularized natural disc reinforced by polycaprolactone, which mimics the natural morphology, and structural, biomechanical and biological property of the TMJ disc. The construct demonstrated good biocompatibility, safety and immunological tolerance both in vitro, and in a rat subcutaneous model. During 6 months implantation in an allogeneic rabbit TMJ disc reconstruction model, the disc prosthesis maintained its integrity, collagen fiber-orientation, mechanical property, joint structural stability and prevented articular cartilage and bone from damage. Furthermore, the “upgraded” disc prosthesis obtained from decellularized porcine disc was implanted into a goat TMJ disc reconstruction model. The xenograft prosthesis, with strength and viscoelasticity similar to a natural TMJ disc, was able to restore the structure and function of TMJ up to 20 weeks. These results demonstrate the translational feasibility of an allogeneic or xenogeneic decellularized disc prosthesis for treatment of advanced TMJ disc-related diseases.

Po2_2.33

Fibercoll-Flex-N® in Bone Tissue Engineering: A new formulation of fibrillar collagen and Hydroxyapatite Bioink for Functional Bone Regeneration

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Abstract

Bone tissue engineering (BTE) aims to induce new functional bone regeneration via the synergistic combination of biomaterials, cells, and factor therapy. Natural bone is an organic and inorganic composite constituting of mineralized collagen fibrils, which serves as the second level of its seven hierarchical levels¹. The main inorganic component in these tissues is nanosized hydroxyapatite. Its crystal structure ascribes to the hexagonal crystal system, which enables it with structural and compositional flexibility and stability². This study explores the use of Fibercoll-Flex bioinks®, composed of pure type I collagen fibers, in combination with hydroxyapatite to create a natural 3D scaffold for BTE applications. The composite material mimics the natural bone structure, enhancing its potential for successful integration.

Three different concentrations of hydroxyapatite were mixed with Fibercoll-Flex-N® bioink to create four distinct formulations: 4% collagen (Control), 4% collagen with 2% hydroxyapatite, 4% collagen with 4% hydroxyapatite, and 4% collagen with 8% hydroxyapatite. Rheological properties, including elastic modulus, viscous modulus, and viscosity, were measured using a Haake Mars 40 rheometer. Bioprinting was performed using a BioX printer (Cellink) to produce various constructs, including cylinders and gyroid cubes, under specific conditions of pressure and speed. Cell viability was assessed using Live/Dead® viability kit.

Significant differences in mechanical properties were observed among the four formulations tested. Higher hydroxyapatite concentrations resulted in bioinks with increased elastic modulus and viscosity compared to the control bioink (Fibercoll-Flex-N® at 4%). These findings suggest that adjusting hydroxyapatite concentration can optimize the mechanical properties of the resulting bioink. High cell viability was observed at both day 1 and day 7, validating the potential of combining the Fibercoll-Flex® bioink with Hydroxyapatite for BTE applications. Additionally, a new freeze-dried scaffold made from GMP grade fibrillar collagen bioink together with hydroxyapatite has been tested for medical devices and tissue engineering applications.

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Po2_2.34

Amorphous calcium phosphate with incorporated glutamic acid enhances bone regeneration.

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Abstract

INTRODUCTION. Bone tissue engineering aims to replicate natural bone's structural and functional properties to promote regeneration. Understanding the effects of implantable materials on healing is crucial. Metabolites, as key regulators of cellular processes, have the potential to modulate tissue regeneration actively. Metabolomics, the comprehensive analysis of small molecules within biological systems, provides insight into the metabolic pathways involved in bone repair. This study used a rat model to identify key metabolites involved in bone regeneration and developed amorphous calcium phosphate (ACP), incorporating these metabolites to enhance healing.

METHODS. A critical-size calvarial defect model in rats was used to study systemic metabolic changes during bone healing. Metabolites identified through bioinformatics analysis were evaluated for their effect on mineralization in MC3T3-E1 pre-osteoblasts. ACP and glutamate-incorporated ACP (ACP-G) were synthesized from calcium glutamate and trisodium phosphate and characterized using X-ray diffraction (XRD) and scanning electron microscopy (SEM). The ACP-G was tested in vitro for osteogenesis, metabolic activity, alkaline phosphatase (ALP) activity, and expression of osteocalcin and osteopontin. ACP-G, ACP, and Bio-Oss® were implanted in vivo to compare bone regeneration and assessed via histological staining and micro-computed tomography (μCT).

RESULTS. Metabolomic analysis of rat serum revealed decreased glutamine and glutamate levels during bone healing. Supplementation with glutamine or glutamate enhanced mineralization in vitro. ACP-G, consisting of ~40 nm amorphous particles, showed sustained glutamate release over three days. Cells cultured on ACP-G exhibited significantly increased expression of osteopontin and osteocalcin and improved mineral deposition (Alizarin Red staining) compared to ACP. Metabolomic profiling revealed enhanced energy metabolism, especially in the TCA cycle. In vivo, ACP-G promoted increased bone formation and integration compared to ACP and Bio-Oss®.

DISCUSSION & CONCLUSIONS. Systemic metabolomic profiling revealed key metabolic shifts during bone regeneration, particularly the downregulation of glutamate and glutamine. ACP-G enhanced osteogenic activity and energy metabolism, likely supporting mitochondrial function and compensating for anaerobic glycolysis. Using metabolomic data to guide biomaterial design offers a promising strategy for improving bone healing and highlights the potential of metabolite-functionalized materials in regenerative medicine.

Po2_2.35

Development of Antibacterial Bone Cements Based on Calcium Phosphate Composites for the Treatment of Bone Infections

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Abstract

Bone infections remain serious complications following orthopedic surgery. Conventional treatment typically relies on the systemic administration of antibiotics, which often fails to deliver adequate drug concentrations at the infection site and may cause undesirable systemic side effects. An emerging alternative involves using calcium phosphate cements (CPCs) as local drug delivery systems. CPCs can be loaded with antibiotics and implanted directly at the infected site, enabling sustained and localized drug release. This strategy enhances local antibiotic concentrations, minimizes systemic toxicity, and may help reduce bacterial resistance.

This study aims to develop antibacterial bone cements from calcium phosphate, calcium carbonate, bioactive glass (BG), sodium alginate (Alg), and ciprofloxacin (Cip), for bone defect filling and the localized treatment of bone infections. The effects of Cip, BG, and Alg on the physicochemical and mechanical properties of the cements, including microstructure, chemical composition, setting time, cohesion, injectability, and compressive strength, were systematically evaluated. In addition, in vitro drug release kinetics, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, cytotoxicity, and inflammatory responses were investigated.

The results demonstrate that BG and Alg significantly influence the cement's characteristics, including cohesion, injectability, setting behavior, and the interaction and release profile of ciprofloxacin. Notably, the incorporation of BG and Alg improved both the rheological and mechanical properties of the antibiotic-loaded cements. The ciprofloxacin release profiles varied depending on the composition, with released concentrations reaching levels comparable to the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *S. aureus* and *E. coli*. Antibacterial assays confirmed that the Cip-loaded formulations effectively inhibited bacterial growth. Moreover, biodegradation tests in phosphate-buffered saline (PBS) revealed that BG and Alg enhanced the degradability of the reference cement. Finally, in vitro studies confirmed the biocompatibility of the formulated cements, showing no cytotoxic or inflammatory effects on human peripheral blood mononuclear cells (hPBMCs).

Po2_2.36

The wear assessment of a hip implant comprised of a silicon nitride femoral head paired with UHMWPE cup

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Abstract

Introduction: Silicon nitride (Si_3N_4) has superior mechanical properties, good resistance to scratching and biocompatibility, making this material a potential candidate for use in hip implants. The use of Si_3N_4 is useful to overcome Metal hypersensitivity (MHS), especially to stainless steel and cobalt-chrome alloy. The long-term survival of hip implants is influenced by the wear resistance of the bearing parts made of ultra-high molecular weight polyethylene (UHMWPE). However, the wear behavior of this ceramic material paired with UHMWPE become an important issue to demonstrate the safety and efficacy of the orthopedic device.

Methods: Si_3N_4 were produced using Hot Isostatic Pressing (HIP), as early described by Maru et al. (2015). As a reference product, commercially available delta alumina femoral heads were also tested. Both were paired with UHMWPE cups (28 mm diameter) A hip joint simulator (AMTI) applied the required force and angular motions (flexion/extension, abduction/adduction, and internal/external rotation) recommended in ISO 14242-1. The wear tests were conducted up to 2.5 million at a frequency of 1 Hz. Calf serum with a protein concentration of (30 ± 2) g/L was used as lubricant, and replaced every 0.5 million cycles. A Coordinate Measuring machine (Mitutoyo) and a profilometer (Taylor-Hobson) were used to measure the depart of sphericity and roughness (Ra) of the femoral heads, respectively. The wear rate of the UHMWPE cups was determined as the slope of the cumulative mass loss as a function of the number of test cycles (ISO 14242-2). An optical microscope (Carl Zeiss) with 5x objective lens was used to assess the worn surfaces of the UHMWPE surface.

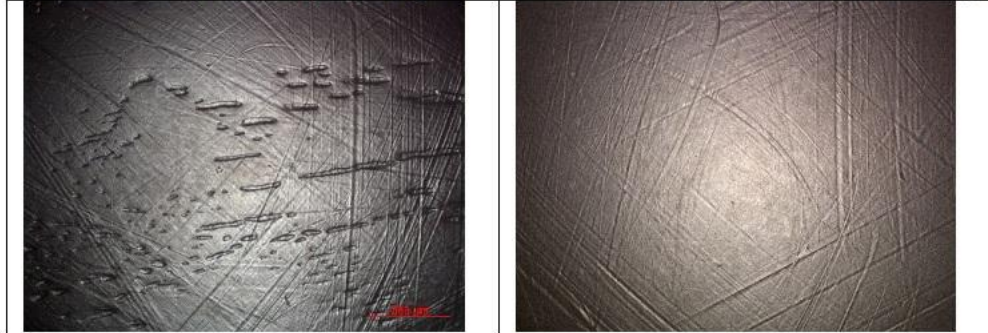
Results: Table 1 summarizes the initial dimensional measurements of the femoral ceramic heads and wear rate of the UHMWPE. A high wear rate was observed for the UHMWPE paired with Si_3N_4 compared to the cups tested against delta alumina. This was assumed to be caused by the high depart of sphericity and roughness (Ra) of the Si_3N_4 femoral heads.

Table 1: Dimensional measurements and wear rates of the femoral head components.

	Sphericity (μm)	Ra (μm)	UHMWPE wear rate ($\text{mg}/10^6$)
Si_3N_4 – sample #1	18.33	0.869	25.32
Si_3N_4 – sample #2	12.12	0.865	12.94
Alumina - sample #1	1.93	0.042	10.21
Alumina - sample #2	2.82	0.048	11.52

Both UHMWPE cups that articulated against Alumina and Si_3N_4 showed a scratched bearing surface, with the removal of the original machining marks (Figure 1).

Figure 1: UHMWPE bearing surface paired against silicon nitride (left) and delta alumina (right).



Conclusion: Improvement in the geometrical characteristics of the Si_3N_4 femoral head can make this material a promising alternative material as bearing articulating surface in hip joints when paired with UHMWPE cups. Use of Si_3N_4 did not introduce different wear mechanisms.

Po2_2.37

Cell-guiding Hydrogel Biomaterials for Bone Organoid Formation.

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Abstract

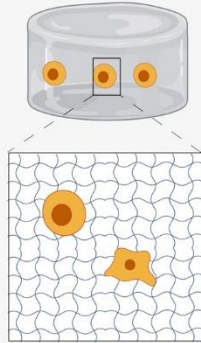
Organoids are tiny 3D tissues grown in the laboratory. Several stem cell-derived organoid systems of human body parts (e.g., intestine, brain, optical cup) have emerged for applications in disease modeling, drug testing and transplantation. However, there is still limited success in generating human organoids for the skeletal system. The major challenge lies in replicating the structural and functional complexity found in bones across several length scales. Inspired by the soft osteoid tissue at the early stage of bone development, we have engineered cell-guiding hydrogel biomaterials with tailored pore architecture that are cell-friendly, biodegradable and conducive to 3D cell growth and osteogenic differentiation.^[1-2] To this aim, we prepared pore-forming hydrogels via (photo)polymerization-induced phase separation (PIPS) technique. These hydrogel biomaterials are fully synthetic and modular in design, combining cell-scale pore sizes (5-40 microns), biodegradability to matrix metalloproteinases, and fibronectin-derived adhesive ligands. When embedded in these hydrogels, human mesenchymal stromal cells and bone-forming osteoblasts can rapidly spread and remodel the matrix to form 3D interconnected functional networks within 24 hours. Our static 3D *in vitro* cultures emphasize that matrix biodegradability plays a key role in regulating osteogenic differentiation, collagen secretion and matrix mineralization. Using a microfluidic device, we successfully generated human bone organoids on chip under fluidic stimulation. Perfusion-induced fluidic shear stress can anabolically stimulate the cells and enhance the osteogenic potential of bone organoids. By mimicking the native osteoid tissue in skeletal development, these cell-guiding hydrogel biomaterials may find new applications in scaffold-directed *in situ* tissue regeneration.

[1] Müller, M.Z., Bernero, M., Xie, C. *et al.* Cell-guiding microporous hydrogels by photopolymerization-induced phase separation. *Nat Commun* **16**, 4923 (2025). <https://doi.org/10.1038/s41467-025-60113-9>

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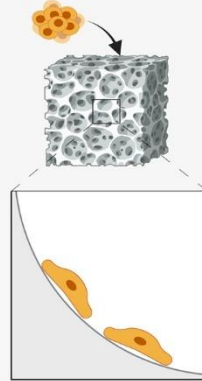
**Conventional biomaterials
to grow bone cells in vitro**

with nanopores
(5 - 100 nanometres)



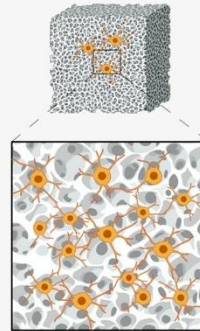
**Bone cells
grow slowly**

with macropores
(100 - 600 micrometres)



**Bone cells
grow flat**

**New hydrogel
with micropores**
(5 - 20 micrometres)



**Bone cells quickly form
a three-dimensional network**

Po2_3.01

Arthroscopic debridement enhanced by intra-articular antibiotic-loaded calcium sulphate beads for septic arthritis of a native knee following iatrogenic joint injection: a case report.

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Abstract

Septic arthritis (SA) represents an orthopedics urgency and mainly affects the knee joint. Due to its devastating effects on cartilage, immediate management is crucial. SA is characterized by an annual incidence of 2 to 10 cases per 100,000 individuals, with mortality rates fluctuating between 0.5% and 15%, with a substantially higher mortality rate observed in older people (15%) in contrast to younger cohorts (4%). The etiology of septic arthritis is multifactorial: a spectrum of Gram-positive and Gram-negative bacteria can contribute to the development of this condition, especially *Staphylococcus aureus*. The treatment involves urgent (arthroscopic or arthrotomic) debridement associated with adequate antibiotic therapy. Intra-articular antibiotic carriers can also be used to increase their local concentration and effectiveness. The case of a 67-years-old woman affected by knee SA from methicillin-susceptible *S. aureus* is presented. She was treated with an arthroscopic debridement enhanced by intra-articular antibiotic-loaded calcium sulphate beads, together with antibiotic therapy. At 2-year follow-up, the infection has been eradicated and the patient fully recovered. This is the first description, to our knowledge, in English Literature of the use of antibiotic-loaded calcium sulphate beads as an adjuvant in the surgical treatment of SA of a native knee joint.

Po2_3.02

Regulatory Role of Mycobacterium tuberculosis Secreted Protein ESAT-6 in Osteoclast Differentiation

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Abstract

Purpose. This study investigates the biological and toxicological effects of ESAT-6, a protein secreted by *Mycobacterium tuberculosis*, focusing on its role in bone marrow-derived macrophage (BMDM) differentiation into osteoclasts, a key process in bone resorption. Specifically, it explores the interaction between ESAT-6 and CMPK2, a protein critical for mitochondrial DNA synthesis and energy metabolism, to elucidate the molecular mechanisms underlying bone degradation.

Methods. The cytotoxicity of ESAT-6 on BMDMs was assessed using cell viability (CCK8) and DNA synthesis (EDU) assays. Osteoclast differentiation was evaluated using morphological (TRAP staining, F-actin organization) and genetic (qPCR) markers, focusing on gene expression changes on the third day post-treatment. Immunoprecipitation-mass spectrometry (IP-MS) and co-immunoprecipitation (CO-IP) identified and confirmed ESAT-6 interactions with CMPK2. Transcriptomic and metabolomic analyses were performed to explore CMPK2's role in mitochondrial dynamics and energy metabolism during osteoclast differentiation. The effect of CMPK2 inhibition on ESAT-6-induced osteoclastogenesis was tested using NDGA, a specific CMPK2 inhibitor.

Results. ESAT-6 exhibited concentration-dependent cytotoxicity on BMDMs, significantly reducing cell viability and DNA synthesis at higher levels. It promoted robust osteoclast differentiation, as evidenced by increased TRAP activity, F-actin reorganization, and upregulation of osteoclast-specific genes, peaking on day three. Proteomic analysis identified CMPK2 as a critical downstream effector of ESAT-6, with CO-IP confirming their direct interaction. Transcriptomic and metabolomic profiling revealed CMPK2's role in regulating mitochondrial DNA synthesis and energy metabolism, essential for osteoclast maturation. Inhibition of CMPK2 by NDGA significantly blocked ESAT-6-induced osteoclast differentiation.

Conclusion. This study highlights ESAT-6's pivotal role in promoting osteoclastogenesis by interacting with CMPK2 and influencing mitochondrial function. These findings provide valuable insights into the mechanisms of *Mycobacterium tuberculosis*-induced bone degradation and suggest potential therapeutic targets to mitigate osteoclast-related bone loss. Future research will focus on further delineating CMPK2's role in bone metabolism and identifying additional ESAT-6 protein interactions involved in disease progression.

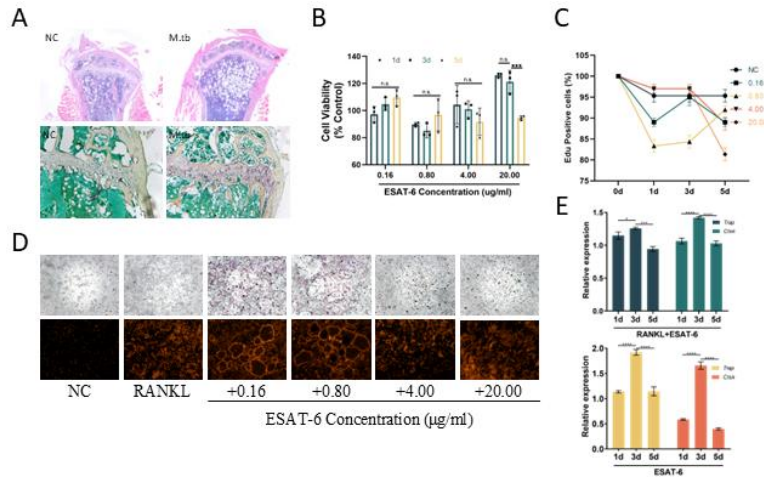


Fig.1 ESAT-6 can promote osteoclast differentiation under the stimulation of low-dose RANKL.

A H&E and TRAP staining of tibiae from normal mice and tuberculosis mice.
 B CCK-8 was used to assess the toxicity of ESAT-6 at different concentrations on macrophages at various time points.
 C EdU was used to assess cell proliferation in macrophages stimulated with different concentrations of ESAT-6 at various time points.
 D TRAP and F-actin staining were used to assess the ability of different concentrations of ESAT-6 to promote osteoclast differentiation.
 E qPCR was used to analyze the expression levels of osteoclast-specific genes at different time points following ESAT-6 stimulation.

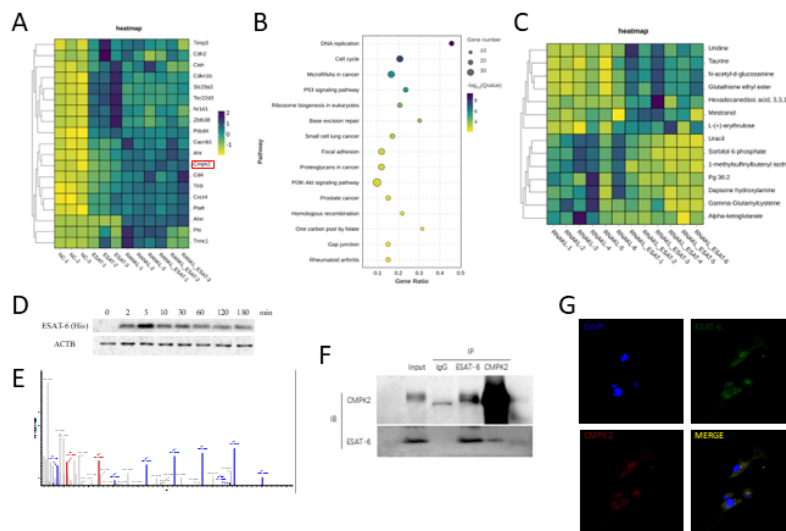


Fig.2 ESAT-6 promotes osteoclast differentiation by binding to CMPK2.

A Transcriptomic analysis suggests that CMPK2 is upregulated during osteoclast differentiation.
 B KEGG analysis revealed pathway enrichment under the influence of ESAT-6.
 C Metabolomic analysis detected changes in cellular metabolites under the influence of ESAT-6.
 D Western blot was used to detect the cytoplasmic expression of proteins at different time points following ESAT-6 stimulation.
 E Tandem mass spectrometry (MS/MS) suggests that CMPK2 may be a binding partner of ESAT-6.
 F Co-immunoprecipitation (Co-IP) was used to detect the protein-protein interaction between CMPK2 and ESAT-6.
 G Immunofluorescence analysis revealed co-localization of ESAT-6 and CMPK2 in the cytoplasm

Po2_3.03

Characteristics and anatomical location of infected pin-sites

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Abstract

Background. Assessment of more than 2000 pin-sites left the impression that certain anatomical locations and other characteristics of the pins were more likely to develop infection. This clinical experience is common despite it has not been reported in the literature. Larger data materials with each individual pin-site tracked might be the barrier, since previously published literature mostly report pin-site infections counted per patient.

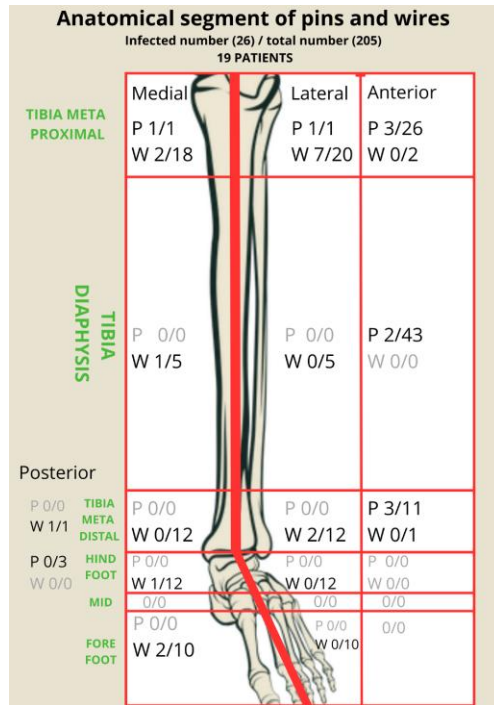
Aim. Report the anatomical location and other characteristics for infected pin-sites for patients with external ring fixators on the lower extremity.

Method. Data was collected in a cross sectional designed study (1). The Modified Gordon classification System (MGS) bed site was used to grade signs of infection. Pus at the pin-site (MGS score 4) was considered a confirmatory sign of infection. Inclusion criteria was first event of a pin-site infection for this descriptive subgroup analysis. Findings are reported as counts and median values of the following characteristics: indication for treatment, symptoms of pain, frame construct, anatomical location and distance to neighbor pins or wires. Anatomical location and distance to neighboring pin-site was evaluated from digital images obtained at the examination day.

Results. 34 (2%) of 1970 pin-sites (83 patients) investigated were infected. 5 patients (8 infected pin-sites) were excluded after first infection event. 19 patients with 26 infected pin-sites (in total 205 pin-sites) were included. Of the 26 infected pin-sites, 17 were Ilizarov-transfixion wires and 9 were thicker bi-cortical inserted half-pins. Indication for treatment was acute fracture (7 patients), prior fracture with no infection (4 patients) and correction/lengthening procedures (8 patients). More than half of the infected pin-sites were in the correction/lengthening group (14 of 26 pin-sites). The majority (14 of 19 patients) had a general sensation of pain with the leg in resting position (median VAS score 3). The specific infected pin-site had a median VAS score of 4. The majority (21 out of 26) of the infected pins sites were located close to a neighbor pin-site (within 2-3 cm). The anatomical most troublesome segment was the proximal tibia metaphysis where 14 of the 26 infected pin-sites were inserted.

Conclusion .We found that the majority of infected pin-sites were located in the proximal tibial metaphysis. A Ilizarov-transfixion wire inserted at the proximal tibia segment laterally

appears to be the most troublesome. The majority of patients having a pin-site infection defined as drainage of pus from the pin-site also experience pain from the pin-site.



	Patients	Pins sites
First event of pin site with infection (MGS 4)	19	26
Indication for treatment		
Acute fracture	7	7
Prior fracture (no infection)	4	5
correction/lengthening	8	14
Symptoms of pain (VAS score)		
General pain in leg/foot with frame resting position (median)	14 (VAS 3)	
Specific pain score for infected pin (median)		14 (VAS 4)
Frame construction		
Wires		17
Pins		9
Close located pin or wire (within 2-3 cm)		15
Other infected pin sites (on same frame on same day)		21
No other infected pin sites (on same frame on same day)		5
Treatment		
Oral antibiotics last week	12	
Start oral antibiotics today	4	
Increased pin care last week	15	
Continue pin care as last week	18	
Wire removal	1	
Removal entire frame construct	1	
Anatomical location, segment		
Tibia proximal metaphysis		14
Tibia Diaphysis segment		3
Tibia distal metaphysis		6
Hindfoot		1
Midfoot		0
Forefoot		2

Po2_3.04

Monitoring Early-Stage Osteoarthritis in a Tissue-Engineered In Vitro Model Using Near-Infrared Spectroscopy

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Abstract

Osteoarthritis (OA) is a chronic degenerative joint disease affecting 600 million people worldwide, characterized by persistent inflammation and the degradation of articular cartilage. The prevalence of OA is increasing due to an aging and increasingly obese population. Currently, there is no cure for OA due to limited understanding of OA pathophysiology, as animal models fail to accurately replicate the human condition. We aim to develop a human stem cell-based model of early-stage OA using tissue engineering (TE). This approach will yield a platform for personalized OA drug screening.

In our previous work, we developed a setup based on near-infrared (NIR) spectroscopy for monitoring TE constructs development without interfering with the culture process [1]. We have also developed novel data analysis pipelines for live estimation of tissue properties from the NIR spectra. In the current study, we aim to develop spectral data analysis pipelines capable of estimating OA disease state.

The model was prepared by embedding human bone marrow-derived mesenchymal stem/stromal cells in gellan gum hydrogels. The constructs were cultured for up to 21 days in chondrogenic differentiation medium to promote cartilage formation. After 14 days of culture, OA was induced using pro-inflammatory cytokines (human recombinant TNF- α , IL-6 and IL-1). NIR spectra were obtained every 24h using our custom setup. We are currently performing biochemical analyses and are analyzing mechanical test results for assessing cartilage formation and detection of OA biomarkers.

Our preliminary analyses show that NIR spectra from constructs treated with pro-inflammatory cytokines are distinct from control constructs (Figure 1). A support vector machines (SVM) model was able to classify the spectra with 76.7% sensitivity and 90% specificity as coming from the OA induced constructs (Table 1).

We plan to enhance our in vitro model further by subjecting it to increased mechanical loading to mimic the mechanical impact of obesity on joints.

Reference

1. Karunarathna, H, Sadeesh, N, et al. (2025). Unpublished.

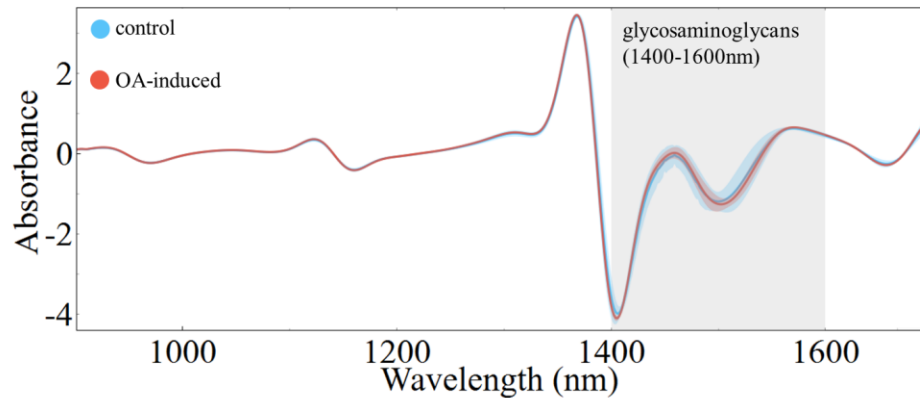


Figure 1: Preprocessed Near Infrared (NIR) spectra. NIR spectral classification was performed based on OA induction with inflammatory factors, with spectra ranging from 900 to 1700 nm. Changes in the spectra were detected for glycosaminoglycans (1400 to 1600 nm, shaded area).

Table 1: The confusion matrices shows that the support vector machines model accurately identified 76.7% of OA-induced samples and 90% of control samples.

		predicted		
		OA	control	Σ
actual	OA	76.70 %	10.00 %	100
	control	23.30 %	90.00 %	100
	Σ	120	80	200

Po2_3.09

Tobramycin and BMP2-loaded collagen scaffold for treatment of fracture related infection while promoting bone healing in a critical size defect model in rabbits.

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Abstract

Introduction. Surgical management of fracture-related infections (FRIs) involves debridement of infected tissues, management of dead space, and systemic antimicrobial therapy. Antibiotic-eluting biomaterials, such as polymethylmethacrylate (PMMA) loaded with gentamicin, are commonly used after debridement but require removal as they may promote antibiotic-resistant infections. This has led to interest in biodegradable antibiotic-eluting biomaterials.

Bone Morphogenetic Proteins (BMPs) promote bone repair but are contraindicated during active infections. This study focuses on developing a scaffold for the dual release of tobramycin and BMP2, allowing for enhanced bone healing while treating the infection in FRIs.

Methods. *In vitro* experiments assessed the release over time of BMP2 and tobramycin from the collagen scaffold. A mouse myoblast cell line (C2C12) was used to assess the osteogenic capabilities of BMP2 (10-200 nM) in the presence of antibiotics (tobramycin vs gentamicin – 10-100 µg/ml) compared to a control.

New Zealand White rabbits ($n=44$) were included in this study. A 5 mm defect was created in the humerus and fixed with a stainless steel plate. During the initial surgery, the 5 mm bone piece was infected with 10^6 colony-forming units (CFUs) of *S. aureus* and placed back in the defect. After 4 weeks, animals underwent revision surgery. Subsequently, the defect was filled with a collagen scaffold with or without BMP2+Tobramycin ($n=9$ per group). Additionally, non-infected animals were included ($n=4$ per group) to compare bone healing outcomes by micro-computed tomography and histology. Animals were euthanized 6 weeks after surgery.

Results. Release of BMP2 and tobramycin from the collagen scaffold revealed a burst release effect over the first 3 days. *In vitro* osteogenic differentiation of C2C12 was significantly inhibited in presence of gentamicin for concentrations above 100 µg/ml ($p<0.0001$), while tobramycin had no effect on alkaline phosphatase expression.

In vivo, local tobramycin cleared infection in all treated animals ($n=5$), while 2 of 5 control animals were culture positive. Over six weeks, bone volume and density were similar across groups (infected and non-infected). However, histology showed increased bridging in the BMP2 groups, both infected and non-infected, compared to controls.

Conclusions

This research developed an antibiotic-eluting scaffold with a dual-release function for treating FRI while promoting bone healing. These scaffolds can be implanted into bone defects after debridement to help eliminate bacteria, and their biodegradable nature eliminates the need for removal, reducing hospital stays and costs. Future studies will evaluate various osteogenic proteins to enhance bone healing in infectious conditions.

Po2_3.12

Assessing diagnostic accuracy: ^{18}F -FDG PET-CT Scans in Low-Grade Infection Detection among Post-traumatic Long Bone Non-unions

Lotte C.A. van der broeck, Anna J.L. Lodewijks, Cristina Mitea, Daan Loeffen, Martijn Poeze, Shan Shan Qiu Shao, Jan Geurts, Taco J. Blokhuis

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Abstract

Introduction. The diagnosis of low-grade infection in post-traumatic long bone non-unions poses challenges due to the absence of clinical signs. This study aimed to assess the diagnostic accuracy of ^{18}F -FDG PET-CT scans for low-grade infection in post-traumatic long bone non-unions.

Material & Methods. A retrospective study included adult patients with a long bone non-union, suspected of infection. All patients underwent ^{18}F -FDG PET-CT scans as the index test before surgical intervention, with peri-operative cultures obtained during surgery serving as the reference standard. Semiquantitative analyses were performed on the standardized uptake value (SUV) measurements obtained from the PET-CT scans. Diagnostic accuracy measures including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Receiver operating characteristic (ROC) curve analysis was employed to evaluate the discriminatory ability of SUV measurements.

Results. The study included 51 ^{18}F -FDG PET-CT scans and cultures from 50 patients with long bone non-unions. The diagnostic accuracy was found to be 0.67 (95% CI 0.44 - 0.87). The PPV and NPV were calculated as 0.79 (95% CI 0.53 - 1.00) and 0.52 (95% CI 0.30 - 0.73), respectively. Quantitative analyses of SUV measurements demonstrating a moderate to low level of accuracy, with all areas under the curve (AUC) values < 0.75 and ROC curves showing a trajectory fairly parallel to the diagonal line.

Conclusion. The findings of this study indicate that the ^{18}F -FDG PET-CT has moderate diagnostic accuracy in detecting low-grade infections in posttraumatic long bone non-unions. A careful interpretation of the scan results is warranted, possibly including the quantitative analysis on tracer uptake as an adjunct. Nevertheless, the diagnostic accuracy in this condition is not as good as in early-onset FRI cases, and this should be taken into account when treating these challenging cases.

Po2_3.14

Evaluation of daptomycin-supplemented antibiotic-loaded bone cement for treating vancomycin-resistant *Enterococcus faecalis* in the *Galleria mellonella* implant infection model

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Abstract

Objective. Periprosthetic infections caused by vancomycin-resistant pathogens, such as vancomycin-resistant *Enterococcus faecalis* (VRE), represent a major clinical challenge. Daptomycin can be incorporated into polymethyl methacrylate (PMMA) cement spacers as a potential treatment. Over the past decade, the prevalence of VRE across Europe has increased markedly (Ayobami *et al.*)^[1]. This study aimed to utilize the *in vivo* *Galleria mellonella* larvae implant infection model to determine the optimal dosage of daptomycin for incorporation into different PMMA cements and evaluate its efficacy.

Material & Methods. Daptomycin (1 g or 2 g) was added to different commercially available PMMA cements, including Simplex T (containing 1 gr tobramycin) and Palacos R+G (containing 0.5 g gentamicin), to produce rod-shaped bone cement implants using a Teflon mould (Zhao *et al.*)^[2]. Control groups included non-loaded Simplex T and Palacos R+G, as well as Simplex P and Palacos R, which contain no antibiotics. To mimic a preventive approach resembling the haematogenous implant infection route, the test specimens were implanted into larvae of the greater wax moth (*G. mellonella*), followed by infection with 250 CFU of VRE DSM13591 after 1 hour. The survival of the larvae was monitored over time, and bacterial numbers in the larval tissue and on the implant surface were determined after 24h. Moreover, these experiments were completed with *in vitro* optical density and inhibition zone tests.

Results. Survival and bacterial burden analysis demonstrated that the addition of 1 g or 2 g of daptomycin was effective in preventing VRE infections. This significantly improved larval survival and reduced bacterial numbers in both the larval tissue and as on the implant surface, regardless of the type of PMMA bone cement used. In contract, Simplex T and Palacos R+G alone did not enhance larval survival and showed outcomes comparable to the antibiotic-free control groups (Simplex P and Palacos R).

Discussion. The incorporation of daptomycin into ALBC successfully prevented VRE infections in an *in vivo* model. These findings underscore the potential of the *G. mellonella* implant infection model as a valuable tool for evaluating the efficacy of ALBC against multidrug-resistant bacteria, thereby accelerating pre-clinical research and advancing strategies to combat periprosthetic infections.

[1] DOI: 10.1080/22221751.2020.1769500. [2] DOI: 10.3390/antibiotics13080692

Po2_3.16

Gel for delivery of antibiotics (GEDAI) demonstrates antibacterial efficacy in sheep models of *S. aureus* orthopedic device-related infections (ODRI)

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Abstract

Introduction/Objectives. Orthopedic device-related infection (ODRI) remains a costly complication often requiring prolonged treatment. Antibiotic-loaded, biodegradable, injectable biomaterials delivering high local antibiotic concentrations could enable single-stage revision surgery, improving outcomes. This study aimed to develop a GEL for Delivery of Antibiotics (GEDAI) meeting these requirements: a) adherence to tissue, not gloves; b) simple clinical implementation; c) delivery of multiple antibiotics. *In vitro* and *in vivo* characterization and antibacterial activity assessment were performed.

Methods. Rheology tested stability and viscoelastic properties of GEDAI loaded with tobramycin (GEDAI-T), gentamicin (GEDAI-G) and gentamicin-vancomycin (GEDAI-GV). Antimicrobial effectiveness was evaluated *in vitro* against methicillin-susceptible or resistant *S. aureus* in planktonic cultures and 24-hour biofilm. Biocompatibility was assessed using human fibroblasts and osteogenic differentiation on human bone marrow stromal cells (hBMSCs, *n*=3 donors). GEDAI formulations were tested *in vivo* through three independent animal studies using Swiss alpine sheep. Two preclinical models were used: the debridement, antibiotic, irrigation and implant retention (DAIR) model with plating osteosynthesis for GEDAI-T and GEDAI-G, and a single stage revision nail model for GEDAI-GV. After *S. aureus* infection periods of 2, 3 or 8 weeks, the sheep underwent revision surgery. GEDAI-T and GEDAI-G were applied over the plate during DAIR revision, while GEDAI-GV was applied in the intramedullary canal before nail replacement in the single-stage model. Control groups received only systemic antibiotics. Thirty sheep were included across the studies (2 weeks infection: *n*=5 GEDAI-T and *n*=5 control, 3 weeks infection: *n*=5 GEDAI-G and *n*=5 control, 8 weeks infection: *n*=5 GEDAI-GV and *n*=5 control). The studies were approved by the Cantonal authorities in Graubünden, Switzerland.

Results. GEDAI demonstrated shear thinning and elastic recovery, indicating good flowability and shape retention. Tack tests showed preferable adherence to metal and tissues over gloves. *In vitro* assays revealed no adverse effects fibroblast viability or hBMSC osteogenic differentiation. Significant reductions in MSSA and MRSA were observed in both planktonic culture and biofilms after exposure to GEDAI-T, -G or -GV compared to control (*p*<0.001). *In vivo* GEDAI-T and -G reduced bacterial counts in DAIR models (3/5 and 1/5

culture positive sheep in the GEDAI-T or GEDAI-G treated animals, respectively), and GEDAI-GV achieved a complete infection eradication in the single-stage revision model.

Conclusions. Local antibiotic application can enhance ODRI treatment outcomes, and in this context, antibiotic loaded hydrogels could be used for treatment of musculoskeletal infections. The GEDAI shows promising translation potential in clinical application of ODRI.

Po2_3.17

Using Metabolomics as a Diagnostic Tool for Early Detection of Fracture-Related Infections: Insights from a Pilot Study.

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Abstract

Fracture-related infection (FRI) is a major complication in orthopedic trauma surgery. Diagnosing FRI can be challenging due to its varied clinical presentations, and the limitations of current diagnostic tools. Metabolomics has emerged as a valuable resource for exploring host-pathogen interactions at the level of small molecules. Understanding the role of host metabolism in FRI is essential for identifying specific metabolic pathways involved during infections and for developing diagnostic biomarkers. We used LC-MS-based targeted metabolomics profiling on serum samples from three cohorts: healthy individuals ($n=9$), fracture patients without infection ($n=9$), and FRI patients ($n=21$) with confirmed *Staphylococcus aureus* ($n=6$) or *S. epidermidis* ($n=15$) infection, to compare global metabolic changes.

After median normalization due to different total metabolite concentrations, we found significant differences across all three groups – mainly in amino acids and their derivatives. An increase of tryptophan (FDR 0.001) and a decrease in threonine (FDR 0.007) and arginine (FDR 0.04) were specific for FRI patients compared to both healthy and non-infected fracture patients, highlighting specific metabolic alterations associated with infection rather than fracture. Additionally, a predictive model based on the ratios of alanine to tryptophan successfully differentiated between healthy individuals and FRI patients, while also classifying fracture patients as non-infected.

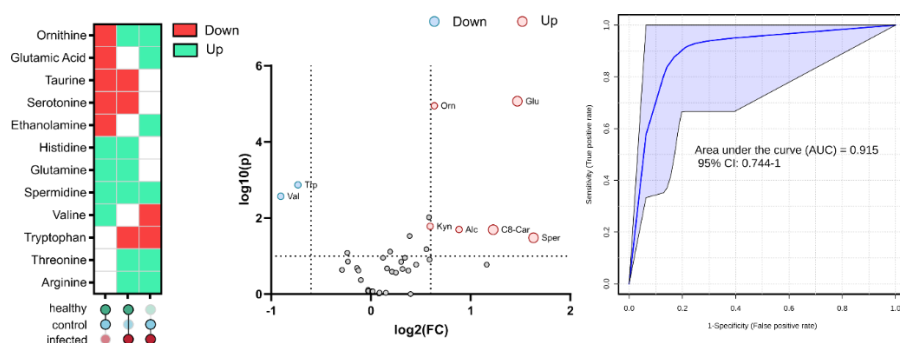


Figure 1. ANOVA results of serum metabolites (left) with a significance threshold of $FDR < 0.05$, volcano plot of changes between fracture and FRI patients with significance threshold $FC > 1.5$ and $p < 0.1$ (middle), and a predictive model using two metabolite ratios to distinguish non-infected from FRI patients (right).

Conclusion. This study represents the first metabolic profiling of serum from patients with FRI. Although the research is limited by the small group size and the need for data normalization to overcome cohort differences, the findings show potential. Serum metabolites could serve as effective biomarkers for FRI in a clinical setting as a simple predictive model was able to distinguish FRI patients from both healthy controls and patients with fracture.

Po2_4.01

Synovial Inflammation Increases Anti-Inflammatory and Anti-Degradative Activity in Mesenchymal Stromal Cells

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Abstract

Mesenchymal stromal cells (MSCs) have emerged as a promising therapeutic strategy for inflammatory and degenerative conditions, particularly musculoskeletal diseases. Synovial inflammation in osteoarthritis contributes to joint damage, pain, and impaired function. This study examines how MSC activity is impacted by synovial inflammation, specifically their potential to reduce inflammation and enhance tissue repair. It was hypothesized that MSCs that encounter inflamed synovial tissue could reduce the tissue's inflammatory profile and associated cartilage degradation.

Synovial tissue was harvested from patients undergoing total knee replacements and conditioned in growth media for 48 hours, after which it was cultured in media with/without MSCs and with/without pro-inflammatory mediators (TNF- α and IFN- γ). After 4 days, the synovial tissue and MSCs were separated, and RT-qPCR was used to examine changes in cell activity.

RT-qPCR revealed significant increases in CD105—an MSC marker—and TIMP3—an inhibitor of tissue remodeling and inflammation—in MSCs when co-cultured with synovial tissue compared to MSCs not exposed to synovial tissue.

TIMP3 upregulation suggests MSCs may be able to mitigate synovial inflammation and cartilage degradation through MMP inhibition. Interestingly, the addition of cytokines attenuated these effects. This suggests that MSCs' anti-inflammatory and anti-degradative potential may be more effective in acute rather than sustained inflammatory environments. This is further corroborated by a significant increase in TGF- β 1 signaling (which uses CD105 as a receptor) in MSCs exposed to only synovial tissue, compared to MSCs not exposed to synovial tissue or cytokines. MSCs exposed to both synovial tissue and pro-inflammatory cytokines, however, did not display a significant increase. Other genes tested include the osteogenic transcription factor RUNX2 and chondrogenic transcription factor SOX9, neither of which showed significant changes. Inflammation-related genes (IL-6 and IL-10) and tissue degradation genes (MMP1 and MMP13) were also tested but failed to produce significant changes relative to MSCs that were not exposed to synovial tissue or cytokines. These findings highlight the importance of understanding MSC responses to varying inflammatory environments in the context of synovial tissue and how this impacts synovial response and cartilage degradation. By elucidating how MSCs adapt to specific inflammatory cues, this study provides valuable insights that could guide the development of targeted, inflammation-sensitive MSC therapies for joint diseases.

Po2_4.02

THE ROLE OF IMMOBILISED WNT16 IN MODULATING ADAPTIVE IMMUNITY AND DIRECTING CARTILAGE REPAIR AND REGENERATION

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Abstract

Osteoarthritis remains a significant clinical challenge, with no therapies to reverse early-stage disease. Stimulating repair to produce mechanically stable cartilage in disease or injury remains an unmet need. This study investigates WNT16, a molecule critical in cartilage maintenance, in an osteochondral repair model.

Spatially localised delivery of WNTs has demonstrated their regenerative potential, and we hypothesise that WNT16 is a candidate for cartilage repair. Previous in vitro studies illustrated the reparative potential of immobilised WNT16 on human cartilage cells in a pro-inflammatory environment. We therefore sought to investigate the mechanisms driving WNT16 healing, in the inflammatory phase of tissue repair.

Using a murine joint surface injury model, WNT16 covalently bound to a polyethylene glycol (PEG) shear gel was locally injected into the defect. Study groups included untreated defects and PEG gel alone. Healing was monitored longitudinally over 8 weeks using MRI and micro-CT imaging, alongside histology, to assess cartilage/bone repair and immune response.

In relation to repair, WNT16-treated defects exhibited superior histological cartilage repair (Pineda=3, $P < 0.04$) with enhanced subchondral bone restoration, cartilage repair signals on MRI and improved mineralized matrix production on micro-CT. Histological analyses confirmed significant bone remodelling and improved cartilage repair in the WNT16-treated group (Fig.1).

In relation to inflammation, at week 1 post-injury in WNT16/PEG-treated mice, immunostaining revealed increased infiltration of pro-inflammatory M1 macrophage markers F4/80^{low}, CD68⁺, CD11b⁺, and pro-repair M2 macrophage markers MERTK^{low} and CD206^{low} cells predominantly lining the deeper part of the defect at sites of nascent tissue formation. In control injuries, few cells expressing these markers were identified at the site of injury. Interestingly, cells found in the synovium proximal to the site of injury were positive for F4/80^{low}, CD11b⁺ and CD206⁺ in WNT16/PEG-treated injuries whilst in controls, these cells were F4/80⁺, CD68⁺, MERTK⁺ and CD206⁺.

To correlate these findings with cartilage repair, further in vitro studies were performed using human blood monocytes polarised towards an M1 macrophage or M2 macrophage

phenotype in the presence of immobilised WNT16. The presence of WNT16 consistently favoured an M1 phenotype and limited M2 differentiation. Interestingly, the secretome of these M1 macrophages enhanced primary human chondrocyte migration and glycosaminoglycan production.

These data demonstrate that localised delivery of WNT16 promotes a more intense M1 macrophage response at the site of injury, which, coupled with effective resolution of inflammation, leads to improved bone and cartilage healing.

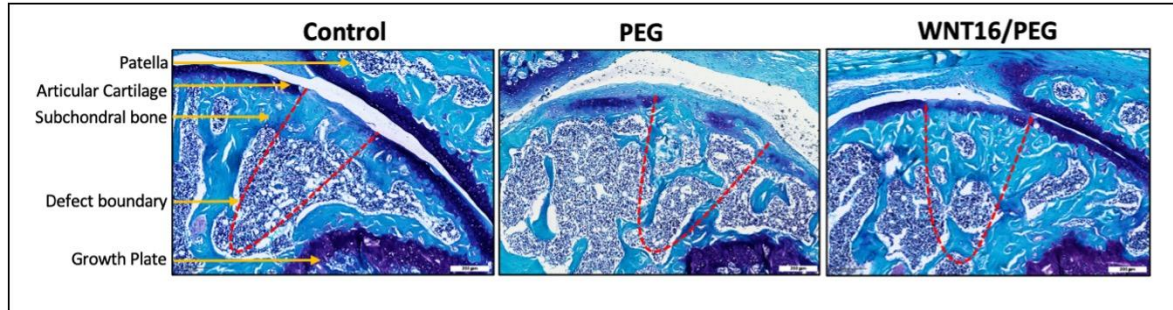


Fig1. Histological representation of osteochondral injury stained with Toluidine blue at 8 weeks.

Po2_4.04

PML3 A Risk Prediction Tool In Patients With Displaced Neck Of Femur Fractures

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Abstract

Background. Displaced femoral neck fractures are common and can be treated with hemiarthroplasty (HA), or total hip arthroplasty (THA). There is no consensus as to which intervention is superior, however THA is offered more frequently to the younger and fitter patients. Sarcopenia is associated with increased complication and mortality rates following hip arthroplasty. The diagnosis of sarcopenia in trauma patients is difficult and the psoas muscle:L3 ratio (PML3) is an alternative muscle mass surrogate marker of sarcopenia. The aim of this study was to assess PML3 as a predictive tool in patients undergoing either HA or THA for a displaced neck of femur fracture.

Study Design & Methods. This single center retrospective observational study was performed with reference to STROBE guidelines. PML3 was measured for all patients with displaced neck of femur fractures included in this study. Psoas major cross sectional area was calculated on an abdominal computer tomography (CT) scan at the L3 vertebra level and normalised to L3 vertebral body surface area. PML3 measurements were compared between HA or THA cohorts and outcome measures included mortality rates, perioperative infections, length of hospital stay, transfusion rates and patient discharge destinations were recorded.

Results. Out of 303 patients with neck of femur fractures between June 2021 and June 2024, 69 (46 female, 23 male) patients were included in the analysis. THA patients had a significantly lower median age (67 vs. 81 years; $P < 0.001$). Postoperative complications were higher in HA patients compared to THA patients (43% vs. 21% OR 1.3, $P = 0.048$). Notably, severe postoperative anaemia was present in 23% compared to 9% of patients in the respective groups. Mortality was also higher in HA patients (14% vs. 6% in THA). PML3 values were significantly different between groups ($P = 0.024$), with higher values in THA (1.84 mm² vs. 1.58 mm²). Patients in the THA group who had postoperative complications had lower PML3 values than the average of HA group (1.25 mm² vs. 1.58 mm²).

Conclusions. Decreasing PML3 is associated with increased morbidity and mortality. Low PML3 scores were present in patients with complications who underwent THA as well as the average of the whole HA group. These results suggest that PML3 can be used as a sarcopenia and frailty index and patients with lower preoperative PML3 scores should be considered for less invasive surgical options such as the HA in the setting of a displaced femoral neck fracture.

Po2_4.05

Mineralization of ATDC5 cells in an *in vitro* endochondral ossification model

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Abstract

Atrophic non-union bone fractures represent a significant challenge in orthopedic medicine. They are characterized by impaired healing due to aberrant endochondral ossification (EO). The development of an *in vitro* mineralization model that accurately recapitulates the EO process is essential. The ATDC5 cell line, derived from mouse teratocarcinoma cells, has been used for studying specific phases of *in vivo* EO. Extracellular matrix (ECM) mineralization has not been extensively described in ATDC5 models. The current study aims to develop an ATDC5-based *in vitro* model that simulates the cartilaginous ECM matrix mineralization phase during EO.

ATDC5 cells were differentiated for 10 days in differentiation medium (DMEM/F12 supplemented with 5% FCS, 1% P/S, 1% NEAA, 10 µg/ml insulin, 10 ng/ml transferrin and 30 nM Na-Se), followed by 7 days of culture in mineralization-inducing medium containing 3.6 mM CaCl₂ and various concentrations of β-glycerophosphate (BGP; 0, 0.5, 1, 3, 6 or 10 mM). Predetermined time point markers of chondrogenic differentiation and mineralization were assessed using RT-qPCR for gene expression analysis. Additionally, calcium and phosphate ion colorimetric assays quantified calcium and phosphate ion concentrations, respectively.

ATDC5 culture mineralization was assessed using quantitative analysis of calcium and phosphate content. Following mineralization induction, supplementation of 3 mM, 6 mM or 10 mM BGP resulted in statistically significant increased levels of both calcium and phosphate content at days 14 and 17, relative to untreated controls, indicating successful mineralization. With increasing BGP concentrations, an increase in calcium and phosphate content was observed, hinting at a BGP dose-dependent increased degree of mineralization. This increase reached a plateau with 6 mM BGP supplementation. RT-qPCR analysis of day 17 samples revealed differential gene expression of hypertrophic markers in response to various BGP concentrations. Three millimolar BGP supplementation resulted in significant downregulation of *Runx2* and *Alpl* compared to untreated controls, while *Col10a1* expression remained unchanged. However, 6 mM BGP induced a statistically significant upregulation of *Col10a1*, while also downregulating *Runx2* and *Alpl*. Surprisingly, there were no differences in hypertrophic gene expression in cells supplemented with 10 mM BGP.

This ATDC5-based *in vitro* model demonstrates progression towards extracellular matrix mineralization. Supplementation of various BGP concentrations combined with CaCl₂ induced matrix mineralization, as demonstrated by increased calcium and phosphate deposition. This mineralization process was accompanied by alterations in the ATDC5 cellular phenotype, as revealed by gene expression analysis. Current investigations focus on elucidating underlying mechanisms of ATDC5 matrix mineralization and cellular phenotypical changes.

Po2_4.07

A “Lucky” Shoulder Injury

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Abstract

Objective. To report a case of a penetrating shoulder injury in a male patient in his 30s, presenting with specific symptoms following a stabbing incident.

Case report: A male in his 30s was transported to the Emergency Department ten hours after a stabbing on his right shoulder. He reported persistent dull pain and was unable to move his shoulder. Upon examination, the patient’s vital signs were stable. A neatly edged wound of about 2.5cm was observed on his right shoulder accompanied with swelling and bleeding. He had tenderness and limited movement of his right shoulder, and numbness on the dorsal skin of his forearm. The peripheral blood supply and movement of his right upper limb were normal. X-rays and CT scans of the right shoulder were taken which showed a knife remaining inside of his shoulder. The knife penetrated through the acromioclavicular joint and the shoulder joint capsule, entering the anterior chest. He was diagnosed with impalement injury of the right shoulder region with brachial plexus nerve deficits due to foreign body (knife) penetration. Arteriography and venography were used to evaluate the spatial relationship and potential involvement of the foreign body with adjacent arterial and venous structures. Electromyography was applied to assess the brachial plexus function and identify the extent and location of nerve damage. The knife was carefully exposed and removed by surgery. The tip edge of the blade was adjacent to the axillary artery and vein. The blade back was close to the lateral cord of the brachial plexus and the distal musculocutaneous nerve. Postoperatively, the patient received anti-infection therapy to prevent infection. Three months later, the patient returned to the hospital, still experiencing numbness and restricted movement in the right upper limb. This time, further examinations revealed that the blood vessels were intact, showing no signs of further compression or damage. However, the brachial plexus, which had previously been tightly pressed against the knife, now appeared frayed at the edges. The scar tissue influenced the nerve, constraining their movement and preventing the arm from functioning properly. Under surgery, we carefully released the nerves from these restrictive bonds. The patient recovered well after the surgery.

Po2_4.08

Enhancing Diabetic Wound Healing by Tibial Cortex Transverse Transport (TTT): The Role of Monocyte-Dependent Th17 Cell Activation

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Abstract

Background. The current therapeutic methods are not always effective for diabetic foot ulcers (DFUs), and further basic research and innovative therapies are urgently needed. The transverse tibial cortex transport (TTT) surgery is a modification of Ilizarov technique based on the principle of distraction osteogenesis. Clinical studies have shown that TTT surgery can promote bilateral healing of DFUs systematically. However, the cellular and molecular mechanisms by which TTT surgery promotes wound healing remain unclear. T cell plasticity plays an important role in DFU healing. Th17 cell, and its marker, IL-17A, maintains immune homeostasis in skin. Lack of Th17 cell and IL-17A delayed wound healing in diabetic ulcers. Whether the TTT promoted Th17 cell differentiation and IL-17A expression to promote wound healing is unknown. Antigen presenting cells (APCs), like monocytes, macrophages and dendritic cells, provide necessary signals for Th 17 cell activation. In DFU, the downregulation of chemotactic receptors and antigen presenting molecules lead to impaired T cell activation and regeneration process.

Objectives. To verify the role of Th17 cells and IL-17A in wound healing enhanced by TTT surgery. To elucidate the interaction between TTT-immobilized circulatory APCs and Th17 cell

Methodology and Results. Single cell RNA-seq dataset of diabetic foot ulcer (DFU) was scanned. Enrichment analysis of mono/macrophage cluster showed activation of wound healing-related genes and antigen-presenting pathway genes. Cell proportion analysis of T cell cluster showed a unique presence of Th17 cells in healing DFU samples.

Therapeutic effect of TTT surgery in a rat foot ulcer model was determined by the changes of ulcer areas and histological evidence. IL-17A expression in skin and peripheral blood was determined by immunofluorescent staining and ELISA, respectively.

Monocyte activation and migration induced by TTT surgery was determined by flow cytometry. Data showed higher CCR2 expression after TTT, suggesting increased monocyte migration. Immunofluorescent staining showed higher MHC-II expression in skin regeneration after TTT, suggesting enhanced antigen presenting ability.

Conclusion. Collectively, our preliminary findings showed that TTT resulted in a better wound healing outcome through the upregulation of IL-17 expressed by Th17 cells. The migration and the antigen presenting capability of monocytes could be responsible for the TTT surgery-induced Th17 activation.

Acknowledgements. This work was supported by grants from Research Grants Council of University Grants Committee Hong Kong (14121721, T13-402/17-N and AoE/M-402/20) and Health and Medical Research Fund (HMRF) of Food and Health Bureau Hong Kong (09203436 & 08190416).

Po2_4.09

The role of viscoelasticity in shaping pro-inflammatory macrophage phenotypes

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Abstract

The formation of a hematoma marks the initiation of a dynamic healing cascade, characterized by immune cell activation and a robust pro-inflammatory response. This early phase of tissue repair involves not only significant biochemical changes but also dramatic shifts in the mechanical properties of the local niche. In this study, we hypothesized that the viscoelastic properties of the mechanical microenvironment act as critical biophysical cues sensed by macrophages, influencing their phenotype and function during the initial stages of healing.

To test this hypothesis, human monocytes isolated from peripheral blood were differentiated into macrophages and encapsulated in ionically crosslinked alginate hydrogels with either fast-relaxing (viscoelastic) or slow-relaxing (elastic) properties. Phenotypic changes were assessed through surface marker expression and cytokine secretion via flow cytometry after 48 hours. Single-cell RNA sequencing was performed at 24 and 48 hours to investigate transcriptional changes associated with mechanical niche sensing.

Our findings demonstrate that the mechanical niche significantly shapes macrophage phenotype and function. A fast-relaxing environment promoted a pro-inflammatory macrophage phenotype, characterized by increased expression of CD80 and enhanced secretion of pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-12p40, despite downregulated expression of HLA-DR and CD86. Single-cell transcriptomic analysis revealed that macrophages in a fast-relaxing environment upregulated genes involved in glycolytic metabolism, antigen presentation, and TLR4 signaling pathways. In contrast, a slow-relaxing environment favored an anti-inflammatory phenotype, marked by the upregulation of genes associated with oxidative metabolism, ECM remodeling, tissue repair, fibrosis, and the transition to a tissue-resident state.

These results indicate that the sudden change of the mechanical microenvironment plays a pivotal role in directing macrophage polarization towards regeneration already at this very initial stage of fracture. Interestingly, the mechanical niche changes generated a macrophage phenotype distinct from that induced by classical pro-inflammatory biochemical stimuli, such as LPS. Both types could also be found in human ex-vivo samples. Moreover, the upregulation of genes involved in sensing and signaling biochemical cues (e.g., CD14, MIF, CD74, TLR4, and interferon- γ receptors) in the fast-relaxing environment suggests that the mechanical niche may sensitize macrophages to biochemical signals. Together, these findings highlight the unique interplay between mechanical and biochemical cues in macrophage polarization and underscore the critical role of the mechanical microenvironment in shaping immune responses during tissue repair.

Po2_4.10

Investigating the Role of Osteoporosis in Rotator Cuff Tears: Causal Relationships and Shared Genetic Loci

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Abstract

Osteoporosis is a recognized risk factor for rotator cuff tears (RCTs), yet its clinical correlation and causality remain underexplored. This study evaluates the impact of osteoporosis on RCT risk, investigates causality, and identifies genetic correlations to understand underlying mechanisms. Using the UK Biobank cohort (N = 457,871), correlation analyses showed that osteoporosis significantly increased RCT risk in a cross-sectional analysis (adjusted odds ratio [95% CI] = 1.35 [1.22 - 1.49]). A longitudinal subset (N = 178,715) followed for 11 years demonstrated that osteoporosis significantly elevated the risk of RCTs over time (adjusted hazard ratio [95% CI] = 1.71 [1.42 - 2.05]) (**Figure 1A**). To establish causality, four inference methods were applied. Propensity score matching (PSM) and inverse probability weighting (IPW) confirmed a significant association (PSM adjusted hazard ratio [95% CI] = 1.67 [1.36 - 2.04]; IPW adjusted hazard ratio [95% CI] = 1.58 [1.23 - 2.03]). Causal random forest analysis identified a causal effect of osteoporosis on RCTs (average treatment effect [ATE] $\pm$ SE = 0.0075 $\pm$ 0.0011). Survival random forest analysis also revealed a significant causal relationship (ATE $\pm$ SE = -0.0476 $\pm$ 0.0043), indicating that osteoporosis increases the risk of RCTs over time. Finally, Genetic analyses using public datasets explored shared loci between osteoporosis and RCTs. Colocalization analysis identified six candidate loci, including PKDCC rs12996954, which was validated as a shared locus for decreased bone mineral density and increased RCT risk, suggesting a horizontal genetic correlation (**Figure 1B**). However, linkage disequilibrium score regression and Mendelian randomization analyses found no significant genetic association, implying limited shared genetic mechanisms. These findings confirm a causal relationship between osteoporosis and RCTs, emphasizing the importance of osteoporosis management in RCT prevention. The identification of shared genetic loci offers insights into potential pathogenic mechanisms underlying these musculoskeletal disorders.

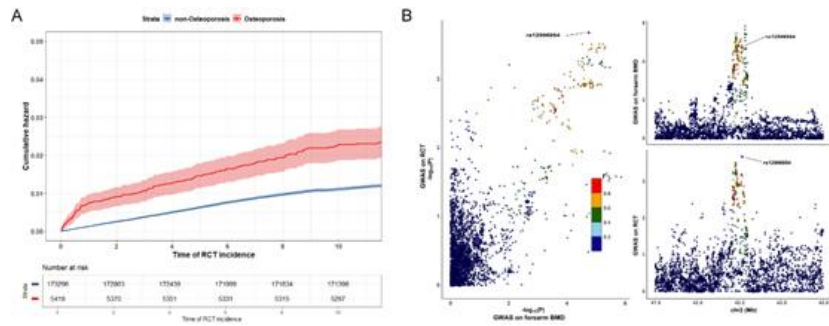
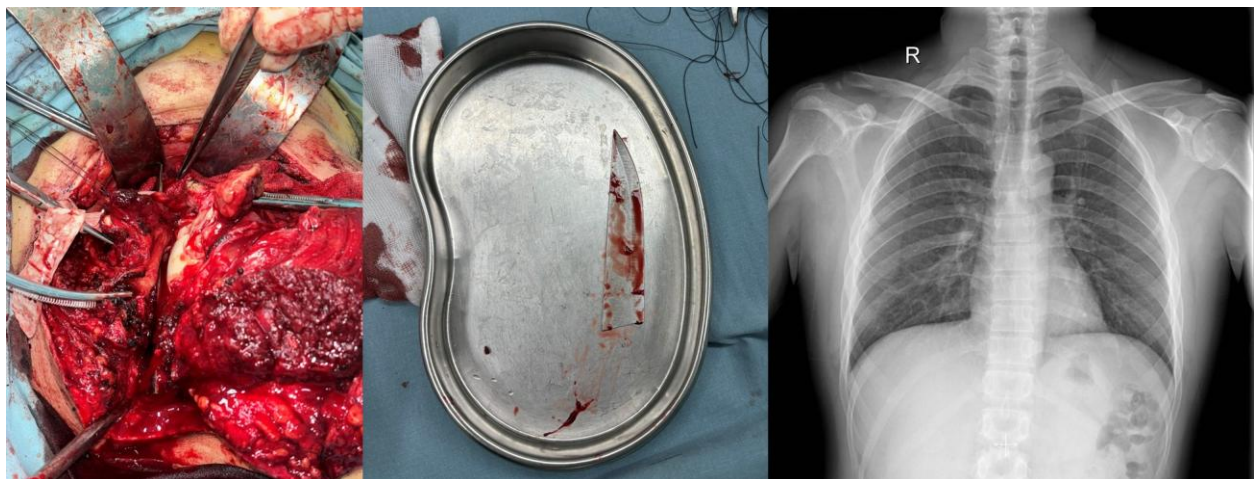


Figure 1. (A) Cumulative risk curves for RCT incidence in the OP group and the control group during 11 years of follow-up. (B) Shared genetic loci PKDCC for BMD and RCTs identified through colocalization analysis.



Po2_4.11

Unravelling the immuno-mechanical dynamics in the early stages of long bone fracture healing

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Abstract

About 5% of long bone fractures fail to heal, resulting in non-union defects for which no reliable treatment strategies currently exist. These cases present significant clinical challenges, and the underlying mechanisms remain poorly understood. To develop effective therapeutic approaches, it is crucial to better understand the biological and mechanical factors driving fracture healing.

Interfragmentary movement (IFM) plays a key role in fracture healing, as the balance between stability and flexibility is essential for proper recovery. Too rigid fixation suppresses IFM and delays callus formation, while excessive flexibility destabilizes the fracture site, hindering healing. While it is known that mechanical cues influence the remodeling phase, their impact on earlier stages, such as the inflammatory response, remains unclear. Bone injury triggers an acute, tightly regulated inflammatory response that is vital for initiating healing. During this phase, immune cells clear debris, recruit skeletal stem cells, and promote tissue repair. Mechanical cues from the environment activate innate immune cells, which release cytokines and chemo-attractants that influence both immune and non-hematopoietic cells. Here, we focus on investigating the role of mechanical environments during the inflammatory phase of healing.

To address this knowledge gap, we examine how different mechanical environments influence the inflammatory phase of fracture healing using a mouse *in vivo* fracture model. We investigate healing under rigid, semi-rigid, and transitioning semi-rigid to rigid fixations. Micro-CT and histology at day 21 show that semi-rigid fixation leads to delayed bone bridging compared to rigid fixation. While excessive ectopic bone growth is observed in the semi-rigid group, bone bridging between the fracture ends remains limited.

To understand the underlying mechanisms during the earlier phases of healing, we use single-cell RNA sequencing on tissue samples collected at days 1, 3, 5, and 7 post-fracture. This approach enables us to map the transcriptomic landscape of immune and non-hematopoietic cells during inflammation under different mechanical conditions, identifying compositional changes, differential cell states, and cell-cell communication.

Our findings will enhance our understanding of the interplay between mechanical and inflammatory factors in fracture repair. This knowledge may contribute to the development of novel therapeutic strategies to improve healing outcomes.

Po2_4.13

EFFECT OF EPIDERMAL GROWTH FACTOR ON PRIMARY WOUND HEALING

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Abstract

Introduction: The skin is the largest organ of our body after the skeletal system. When it comes to surgical clinical practice, it is necessary to make an incision in the skin when a surgical procedure is performed on patients. If the wound lips are closed in a way that they are in full contact with each other, this is called primary wound healing. In case of insufficient attention to the cleanliness of the incision area or if the incision line is closed with tension, openings or infectious processes may be encountered at the wound site. Different suture techniques have been developed to prevent this. In addition to the development of suture techniques, with the advancement of today's medicine, one of the situations sought for a solution in terms of accelerating surgical wound healing in medical treatment has become one of the situations. One of these is EGF (Epidermal Growth Factor). It stimulates cell growth and maturation by binding to EGFR. In our study, we aimed to evaluate the epithelialization process around the wound site by using EGF in the dressing of the incision sites of animals and to examine its contribution to biomechanical durability in skin scar formation.

Method: In our study planned as two groups (n=3), a skin incision of 3 cm length was applied to the right hip region of the rats with the help of a number 10 scalpel. The skin incision area was cleaned with Bathicon solution and sutured with 3/0 prolene suture. Bathicon dressing was applied to the rats in the control groups. Bathicon dressing was applied to the study group with EGF. The study was terminated on the 10th day. A total of 6 animals, n=3 in four groups, were subjected to biomechanical testing.

Results: As a result of biomechanical tests, the durability of scar tissue in the EGF group was measured as an average breaking force of 74 N (Newtons) and an average breaking time of 1.23 seconds (seconds). In the control group, the average breaking force was measured as 61 N and an average breaking time of 0.82 seconds. Both the breaking force and breaking time were found to be higher in the EGF group than in the control group.

Conclusion: We think that EGF primarily contributes positively to wound healing, and that the wound scar tissue is more durable. We think that it should be supported by more experiments to be conducted in the future.

Po2_4.14

Investigating lymphangiogenesis in bone fracture models using a spatial transcriptomic approach

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Abstract

Bone has a remarkable capacity for regeneration after fracture, yet approximately 5% of bone fractures either fail to heal or experience delayed healing. To develop solutions for these patients, an in-depth understanding of bone fracture healing is required.

The role of lymphatic vessels in the context of fracture healing has not been unequivocally solved. In general, lymphatic vessels are involved in transport of inflammatory immune cells. During fracture healing, a correct immune response is required. Our preliminary data using spatial transcriptomics indicate that the lymphatic vessels are present during the embryonic bone development. Therefore, we hypothesize that lymphatic vessels have a function during the inflammatory phase of bone fracture healing.

To explore this hypothesis, we will use the novel open source spatial transcriptomics platform Nova-ST (1). This technology allows for an unbiased, single cell resolution of fracture healing that is resolved in both time and space. Our animal models consist of a healing fracture and a large bone defect model, both using an external plate fixator. Mice will be euthanized at multiple timepoints, therefore, we will be able to capture the evolution of our models at various stages during regeneration. These datasets will allow for the transcriptomic characterization of the regeneration process in both regular fractures and large bone defects in mice. Subsequently, the effect of lymphatic vessels in these models will be assessed.

Taken together, this research aims to improve our understanding of commonly used bone regeneration models and highlight the role of lymphatic vessels. The anticipated results will provide valuable input for the development of targeted therapeutic strategies and computational models.

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Po2_4.16

Therapeutic Application of Recombinant Human Platelet-Derived Growth Factor-B Homodimer and Collagen-Bioceramic Composites for Critically-Sized Non-Union Bone Defects

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Abstract

Introduction: Non-union during healing of bone fractures is a common, yet complicated clinical presentation to treat. Recombinant human Platelet Derived Growth Factor-B chain homodimer (rhPDGF-BB) has been previously shown to promote angiogenesis during healing in the hindfoot and ankle. rhPDGF-BB combined with a bovine type I collagen/ β -TCP matrix (AI) could therefore serve as a viable alternative to autografts in the treatment of non-unions.

Materials/Methods: 2 mm defects were surgically induced in tibiae of New Zealand white rabbits (n=17) and allocated into either – negative control group (Neg CTRL - empty defect followed by 8-weeks of healing), acute treatment group (treatment with AI during primary surgery followed by 4- (AcuSho) or 8-weeks (AcuLng) of healing), or chronic treatment group (ChrLng - no treatment during primary surgery; secondary intervention after 4-weeks with AI injection into defect followed by 8-weeks of healing). Samples were histologically prepared for qualitative and semi-quantitative analysis and imaged using Dual-Energy X-ray Absorptiometry (DEXA) and microcomputed tomography (μ CT).

Results: Histomorphometry and volumetric reconstruction showed delayed healing or non-healing in the negative control group, whereas defects treated with AI in an acute setting yielded bone formation as early as 4-weeks with bone growth appearing discontinuous. At the extended healing time point (8-weeks, acute setting), substantial remodeling was observed with higher degrees of bone organization characterized by appositional bone growth. The chronic treatment group evidenced bone formation and remodeling, with no indication of non-union after treatment with AI. Furthermore, bone growth in the chronic healing group was observed to be continuous with an apparent reduction in the interposed fibrous tissue and an increased presence of osteons, osteonal canals and interstitial lamellae.

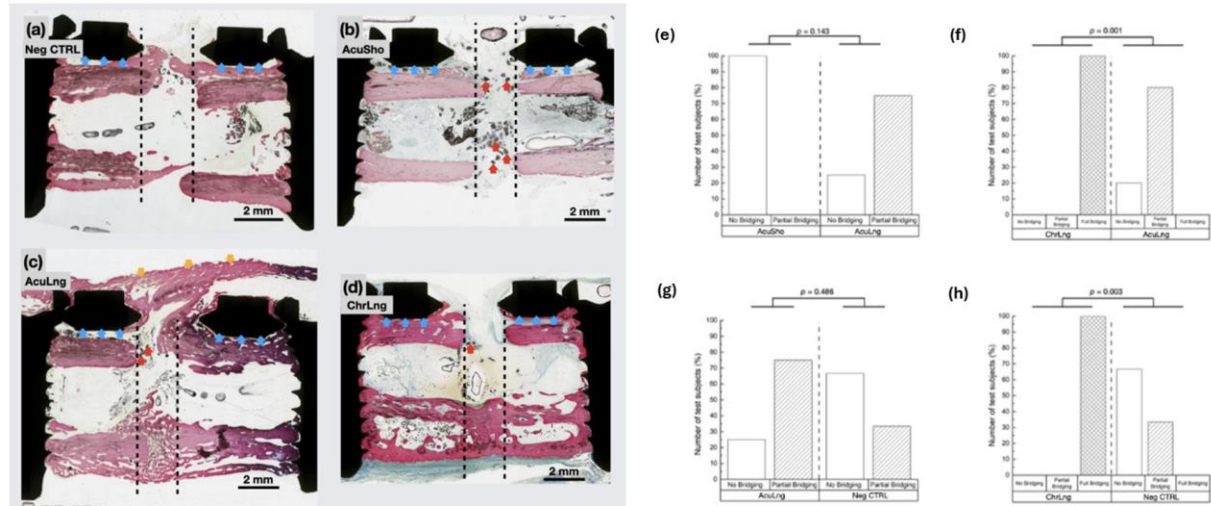


Figure 1: Histomicrographs of (a) Neg CTRL, (b) AcuSho, (c) AcuLng, and (d) ChrLng groups. Dashed black line = induced 2 mm defect, blue arrows = surgical hardware, red arrows = ceramic particles, and orange arrows = stress-shielding. (g-h) Semiquantitative differences in bone bridging between groups, with $p < 0.05$ being statistically significant.

Conclusion: The administration of AI successfully healed bone defects and/or non-unions in this preclinical translational model with increased vascularization and bone remodeling when used in a chronic treatment setting.

Po2_4.17

The impact of medial patellotibial ligament injury on the tension of the medial patellofemoral ligament

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Abstract

Objectives: The medial patellofemoral ligament (MPFL) plays a crucial role in maintaining knee joint stability and motor function, particularly in the prevention and treatment of patellar dislocation. The objective of this study is to evaluate the role of medial patellotibial ligament (MPTL) injury on the tension of MPFL in a cadaver study.

Methods: Three cadaveric specimens were selected for this study. Incisions were made along the medial the patella in each specimen to expose and dissect the MPFL and the MPTL layer by layer, ensuring careful preservation of adjacent structures to maintain anatomical integrity. Biomechanical sensors were utilized to simulate the fixation position of the MPFL graft. In the control group, the MPTL was left intact. Sensors were secured at anatomically determined positions on both the patellar and femoral sides of the MPFL. Tension changes were recorded at knee flexion angles of 0°, 30°, 60°, 90°, and 120°. In the transection group, the MPTL was sectioned, tension changes were again recorded at the same knee flexion angles. In the external force group, after transecting the MPTL, a continuous horizontal outward shear force of 10N was applied to the patella. Tension changes were recorded at different angles using the same method. Data from all groups were collected and analyzed using statistical software to compare the tension differences of the MPFL among the control, transection, and external force groups at various knee flexion angles, thereby evaluating the impact of MPTL integrity on MPFL tension during knee movement. Paired sample t-tests were conducted for the control group

Results: At 90° of knee flexion, the average value of the tension sensor in the control group was (1.47 ± 0.202) N, and that in the transection group was (1.68 ± 0.275) N, with a statistically significant difference ($p < 0.05$). At 30°, 60° and 90° of knee flexion, the average values of the tension sensor in the control group were (1.42 ± 0.076) N, (1.35 ± 0.132) N and (1.47 ± 0.202) N respectively, while those in the external force group were (2.15 ± 0.132) N, (2.00 ± 0.260) N and (1.83 ± 0.284) N respectively, with statistically significant differences ($P < 0.05$).

Conclusion: The integrity of MPTL has influences on the tension of the MPFL at various knee flexion angles. Therefore, it is recommended to consider combined MPTL reconstruction in some cases of patellar dislocation combined with MPTL injury.

Po2_4.20

Comparative spatial analysis of axolotl and mammalian patterning during limb development and regeneration

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Abstract

Despite our ability to heal bone fractures scar-free, the ability of mammals to regenerate amputated skeletal tissue is restricted to the distal 1/3 of the third phalangeal element. Unlike this limited regenerative potential in mammals, the axolotl is a model of unparalleled tissue regeneration, capable of regrowing entire limbs. Within axolotls, limb regeneration requires the contribution of both anterior and posterior (A-P) identity tissue. To dissect out patterning gene regulatory networks occurring during limb regeneration, we analyzed previously published spatial transcriptomics data of an axolotl limb following lower arm amputation. To characterize gradients of gene expression present along a single patterning axis, we developed a new R package known as SpatialTime. Briefly, SpatialTime works by establishing reference lines/spots/tissues and uses Euclidean distances to create a linear, distance-based expression axis across samples. Analysis of known A-P genes showed clear spatial distribution within both the mature and wound epithelium, as well as within the blastema, the critical structure responsible for new limb outgrowth. Interestingly, we noted correlative expressions of 123 genes regulated along both the A-P and proximal/distal (Pr-Di) axes, including the fibroblast growth factor receptor FGFR2 and the WNT regulator WIF1. This data suggests a potential interaction between genes regulating A-P and Pr-Di patterning. The current classical model suggests an A-P boundary defined along the midline of the limb. However, previous work suggests an unequal distribution of A-P gene signatures present along the Pr-Di axis, with more anterior signatures present in the proximal region and more posterior tissue present at the distal end. Here, we propose a revised model known as the McCusker border-shift model, in which the A-P boundary is present off-axis, resulting in decreasing A-P ratios as you move from proximal to distal within the limb. Using our SpatialTime package, we found significantly greater ability to distinguish A-P patterning genes when using the border-shift model as compared to the classical midline boundary. To determine whether this border-shift model was specific for axolotl limb regeneration, we next conducted similar analyses of published spatial transcriptomics data obtained from human limbs during fetal development. Similar to what was observed in axolotl regeneration, expression of A-P genes aligned significantly better within our border-shift model, as compared to the classical midline model. These data suggest a new paradigm in which asymmetric distribution of A-P tissue can provide Pr-Di positional information, supporting limb patterning across species under both developmental and regenerative conditions.

Po2_4.21

Case report: severe subgaleal haematoma associated with methamphetamine intoxication.

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Abstract

Background: Cephalohematoma is a rare extracranial hemorrhagic condition in adults, typically resulting from repetitive mild to moderate head trauma. Its management in adults remains unclear, as no specific guidelines exist for its treatment. While common in neonates, its occurrence in adults is unusual and often associated with coagulopathy.

Objective: This case report aims to describe the presentation, management, and outcomes of an extensive cephalohematoma in a patient with methamphetamine intoxication, highlighting the role of early surgical intervention and multidisciplinary care.

Results: A 43-year-old male with a history of polysubstance abuse presented with a large circumferential cephalohematoma following a methamphetamine-induced crisis, during which he repeatedly struck his head against the floor and walls. On arrival, he exhibited agitation, involuntary movements, and significant scalp swelling. Imaging revealed a cephalohematoma and a displaced nasal bone fracture but no intracranial injury. Prompt surgical drainage of 300 cc of blood, application of a compressive dressing, and prophylactic antibiotics were initiated. Consultation with neurosurgery, ophthalmology, and otorhinolaryngology confirmed that no further surgical interventions were necessary. The patient demonstrated clinical improvement and was discharged with a resolved hematoma and stable neurological status. Despite recommendations, he declined addiction therapy.

Conclusion: This case emphasizes the importance of early surgical management of extensive cephalohematomas in adults, particularly in the context of substance abuse. Given the lack of established guidelines, there is a need for consensus to standardize the management of cephalohematomas in adults. A multidisciplinary approach is crucial in optimizing patient outcomes and addressing underlying risk factors.

Po2_4.22

TrkA⁺ sensory nerve signaling is required to promote digit regeneration

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Abstract

Humans, monkeys, and rodents regenerate the distal end of the digit tip after amputation. After injury, tissue regeneration is coordinated by an intimate structural and functional relationship between fibroblasts and nerve axons, but this process is not well understood. The pan neuronal marker TUBB3 shows extensive innervation prior to amputation along the dorsal and ventral surfaces of the digit. Following amputation, re-innervation occurred along the surfaces of the regenerating bone, eventually extending to the distal tip. ScRNAseq analysis identified the neurotrophin nerve growth factor (NGF) as the primary driver of reinnervation, derived primarily from fibroblasts in early regeneration. To determine the role of nerves in the regenerative process, we used both scRNAseq data derived from the dorsal root ganglia (DRG) (containing the cell bodies of nerves known to innervate the digit), and scRNAseq and spatial transcriptomic data from the regenerating digit. Iterative predictive modeling and histology identified spatial transcriptomic spots likely to contain nerve axons and signaling gradients derived from either all DRG nerves or those derived from nerves expressing TrkA, the high affinity NGF receptor. Consistent with other models, TrkA⁺ nerves promoted cell proliferation in neighboring fibroblasts. In addition, we also noted expression of WNT activation by TrkA⁺ nerves in the nailbed, while the absence of TrkA nerves resulted in TGFβ expression and the mechanotransductive Hippo pathway. To determine the role of TrkA⁺ nerves in regeneration, we TrkA^{F592A} transgenic mice to chemically inhibit NGF/TrkA signaling. TrkA mutant mice showed significantly reduced BV relative to D0 at D17 (CON 0.8342 ± 0.152, TrkA 0.7434 ± 0.056, p<.01) and D21 (CON 1.081 ± 0.202, TrkA 0.7831 ± 0.263, p<.01). Analysis using spatial transcriptomics showed reduced TrkA⁺ nerves, detected by marker gene expression and immunofluorescence, was associated with decreased blastema proliferation and WNT activation. Interestingly, TrkA inhibition resulted in increased innervation of non-peptidergic axons, increasing TGFβ activation and altering mitochondrial morphology through increased fusion and decreased fission, leading to increased expression of transcripts responsive to reactive oxygen species. Our data point to a critical role specifically for TrkA⁺ sensory nerves and identify molecular cascades regulated by nerve subtypes within the regenerating digit.

Po2_5.04

Clinical Application of an Intelligent Walker and Remote Rehabilitation System in Lower Limb Orthopedic Postoperative Rehabilitation

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Abstract

In clinical orthopedics, patients after lower limb surgery routinely require weight-bearing functional exercises to enhance bone healing and limb function restoration. Currently, these exercises are mainly carried out by patients independently after hospital discharge. However, due to the absence of clear guidelines and effective monitoring measures, numerous problems have emerged. Patients often struggle to accurately control the weight-bearing intensity and timing, leading to compromised safety. Additionally, the efficacy of rehabilitation training cannot be promptly reported back, preventing doctors from making real-time adjustments based on patient experiences. Consequently, the scientific validity of the rehabilitation plan cannot be ensured, which in turn impacts the overall rehabilitation outcome. To address these clinical requirements, we have developed an intelligent walker for precise weight-bearing of the lower limbs after orthopedic surgery and a corresponding remote rehabilitation system. This system comprises an intelligent walker and a cloud-based analysis system for lower limb weight-bearing data. After surgery, the patient's attending physician formulates a weight-bearing plan. The patient wears pressure-sensing shoes, and the lower limb weight-bearing training data can be instantaneously displayed on the walker. When the weight-bearing reaches the preset value, the walker emits a distinct alert to guide the patient in performing precise weight-bearing exercises. Upon completion of the training, the patient can provide feedback based on the exercise session. The system records and uploads the training data to the cloud-based analysis system. The background artificial intelligence algorithm continuously optimizes the rehabilitation plan based on the accumulated data and relays it back to the patient's terminal, thereby realizing remote, personalized, and precise rehabilitation guidance. This system has successively completed the prototype fabrication, including the principle prototype, experimental prototype, and engineering prototype, as well as the associated software development. It has obtained the China Class II medical device registration certificate and completed the US FDA registration. Currently, it has been widely promoted and applied in numerous hospitals across 22 provinces and cities in China. Moreover, the first multi-source heterogeneous weight-bearing rehabilitation big data and automatic analysis system has been established, which can offer remote guidance to patients through machine learning. This represents a valuable exploration of new models and methods in remote rehabilitation.

Po2_5.05

Ultrasonic vs. Conventional Saw Bone Cutting in a Rat Tibial Fracture Model: Comparable Healing Outcomes

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Abstract

INTRODUCTION. Ex vivo studies have shown that Ultrasonic bone scalpels cut bone with greater precision. However, the effect of ultrasonic cutting of bone healing has not been evaluated. This study compared an innovative miniaturised ultrasonic bone scalpel and Conventional electrical saw in a rat tibial osteotomy model. The ultrasonic device employed a flexensional configuration with a pre-stressed piezoelectric (PZT) stack and a single metal frame, amplifying blade movement for greater cutting efficiency.

METHODS. Eight adult male Sprague-Dawley rats were divided into two groups: Conventional electrical saw (NS) and ultrasonic (US) cutting. Anaesthesia and analgesics were given, and tibial osteotomies were created using either NS or US bone scalpel (25 kHz at 60 μ m). Continuous saline cooling was applied, and intramedullary fixation was done with an 18G needle. X-rays were taken at multiple time points. All procedures were performed under a UK Home Office License. Bone healing was also assessed using micro-CT imaging and biomechanical four-point bending tests. Additionally, healing was assessed histologically and with fluorochrome bone labelling.

RESULTS and DISCUSSION. At 35 days post-osteotomy, bone mineral density (BMD) and stiffness were compared between the ultrasonic and Conventional electrical saw groups, with contralateral (undivided) limbs served as controls. No significant stiffness and BMD (NS: 0.93 ± 0.08 ; US: 0.98 ± 0.12) difference was observed between groups, although the US group (76.89 ± 25.50 N/mm) exhibited slightly lower stiffness than the NS group (95.66 ± 14.06 N/mm).

Callus index and RUST scores was progressed at a nearly identical rate in both groups, indicating comparable bone healing. These findings suggest that US bone cutting does not negatively impact fracture healing compared to traditional saw methods (Fig 1).

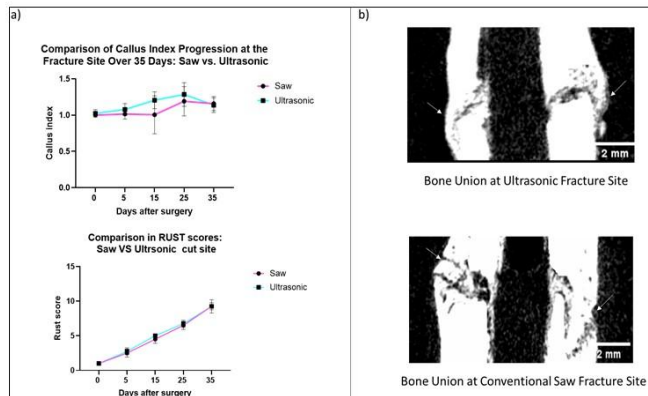


Figure 1: Progression of the callus index and Rust score (a) and Micro-CT image (b) of the fracture site (white arrows) over 35 days at the Normal saw and ultrasonic cut sites displayed comparable results. Data are presented as mean $\pm$ SEM.

Bone formation was visualised using time-specific fluorochromes: tetracycline (day 5, yellow), calcein (day 15, green), and alizarin (day 25, red), outlining the mineralisation timeline. Double-line labelling indicated progressive deposition between days 15 and 25. Both osteotomy techniques supported effective bone regeneration, but the Saw group displayed more advanced remodelling by day 35, with greater presence of osteonal structures (Fig 2a) and collagen maturation, indicating a higher degree of structural maturation. Both groups exhibited similar labelling patterns, with comparable area and perimeter measurements of newly deposited bone (Fig 2b).

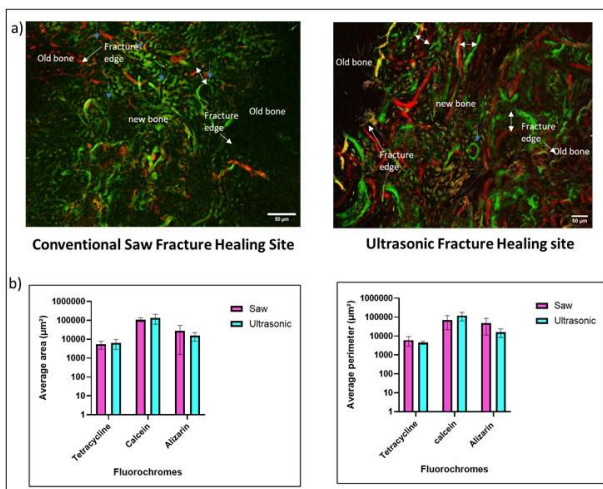


Figure 4: Fluorescence photomicrograph of the fracture site and comparison of fluorochrome measurements in newly deposited bone. a) Triple fluorochrome labelling (tetracycline, calcein, alizarin) revealed sequential bone formation at ultrasonic fracture sites. Multicoloured osteons indicated active remodelling, with distinct deposition phases shown by separated fluorochrome lines. b) Fluorochrome-labelled bone area and perimeter were measured at 5, 15, and 25 days post-surgery, comparing ultrasonic and normal saw cuts. Data are presented as mean $\pm$ SEM from 4 rats per group, each with a healing tibial fracture.

CONCLUSION. Ultrasonic cutting has demonstrated higher precision in ex vivo bone samples, but it was not known whether it was deleterious to bone healing. This study has shown that ultrasonic cutting does not impair fracture repair.

Po2_6.01

Exploiting Mechanical Cues to Enhance Mesenchymal Stem Cell Differentiation for Modern Orthopaedic Applications

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Abstract

Introduction. The trabecular bone's microarchitecture and composition, which vary with age and location, determine bone strength and fragility [1]. Bone adapts its microarchitecture and composition in response to mechanical stimulation [2]. In bone healing, mesenchymal stem cells (MSCs) are recruited, and their osteogenic differentiation is influenced by biochemical and mechanical factors [3, 4]. This study aimed to evaluate the performance of an upgraded bioreactor-based platform utilizing novel 3D-printed biomimetic scaffolds.

Methodology. Scaffolds. 3D-printed polylactic acid (PLA) scaffolds (referred to as P3S3) designed to mimic the human trabecular bone architecture in physiological conditions and to resemble the morphological features of bone, specifically ulna, tibia, and femur (**Fig. 1A**).

Cells. Bone marrow-derived MSCs (BMSCs) seeded onto the P3S3 scaffolds and cultured for 14 days under various mechanical stimuli.

Bioreactor. A bioreactor platform that allows for the application of various mechanical stimuli, including fluid flow-induced shear stress induced by direct perfusion with flow rates (Q) between 0.006-24 mL/min [5], and cyclic hydrostatic pressure induced by the intermittent closing of a solenoid-actuated pinch valve with periods (T) between 0.25 to 30 seconds and duty cycles (DC) between 0 to 100% (**Fig. 1B & Fig. 1C**).

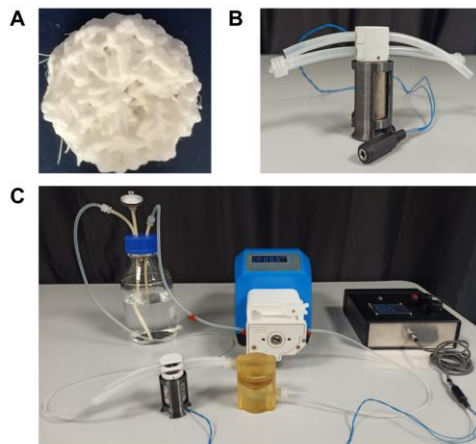


Figure 1. (A) Biomimetic PLA scaffold. (B) Solenoid-actuated pinch valve. (C) Solenoid-actuated pinch valve placed downstream the culture chamber of the bioreactor.

Results & Discussion. Results demonstrated enhanced cell viability (**Fig. 2A**) and osteogenic differentiation under rehabilitation program 1 (RP1) (Q = 0.3 mL/min, T = 10 s, DC = 70%) compared to direct perfusion (D) (Q = 0.3 mL/min) (Fig. 2B). Furthermore, hydrostatic pressure's application significantly upregulated gene expression associated with mechanotransduction, indicating its crucial role in bone tissue engineering (**Fig. 2B**).

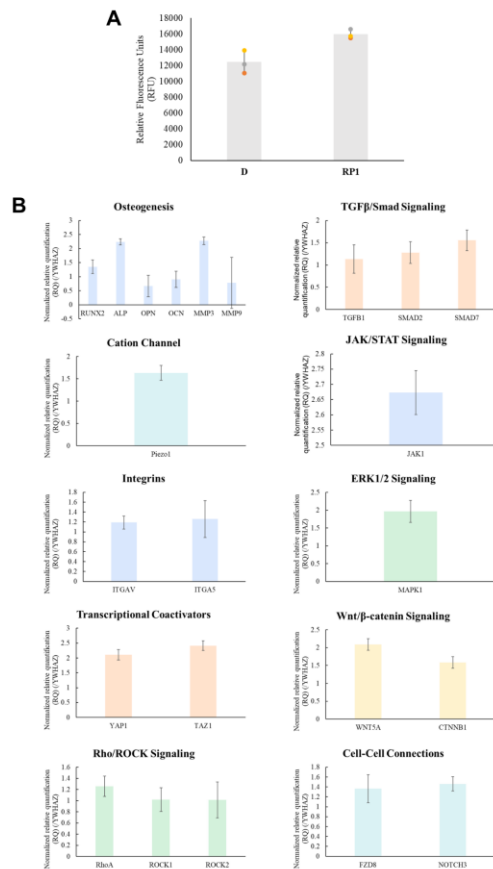


Figure 2. Metabolic activity (**A**) and gene expression (**B**) were compared in cells cultured under RP1 (shear stress + hydrostatic pressure) vs. shear stress alone (D).

Conclusion. This study demonstrates the enhanced capabilities of the upgraded bioreactor platform and P3S3 scaffolds in creating more physiologically relevant *in vitro* models for bone biology research.

“Study carried out within the BIGMECH project – funded by European Union – Next Generation EU within the PRIN 2022 program (D.D. 104 - 02/02/2022 Ministero dell’Università e della Ricerca)”

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Po2_6.02

Dexamethasone-Induced Gene Expression Dysregulation in Osteogenic Differentiation and Inflammation of Human Mesenchymal Stromal Cells

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Abstract

Dexamethasone is used to promote osteogenic differentiation and mineralization of human bone marrow mesenchymal stromal cells (BMSCs). However, its clinical side effects include increased osteoblast and osteocyte apoptosis, and increased osteoclast survival, leading to osteoporosis and higher fracture risk. Our previous research showed that dexamethasone promotes osteogenesis in human BMSCs by downregulating SOX9, but it also upregulates *PPARG*, promoting adipogenic differentiation in some cells. Other studies indicate that dexamethasone reduces mineralization and osteogenic marker expression while increasing osteoclastogenic factors and adipogenic markers. The overall effects of dexamethasone on human progenitor differentiation are contradictory and not fully understood, prompting further investigation using sequencing approaches.

Human BMSCs (n=3 donors) were induced to osteogenic differentiation in a medium containing DMEM, ascorbic acid, β -glycerophosphate and different concentrations of dexamethasone or (+)-ZK216348, a synthetic glucocorticoid inducing only transrepression. After DNase treatment, cDNA libraries were prepared from day 7 RNA samples using Oxford Nanopore Technologies (ONT) protocols (SQK-PCB109). RNA sequencing (RNAseq) was performed with an ONT R9.4 flow cell, and data processing included basecalling, barcoding, quality assessment, and differential gene expression analysis. qPCR analysis of differentially expressed genes was performed for validation purposes both in the sequencing cohort and in additional donors (n=3). From the second cohort of donors, day 7 conditioned medium was collected and used for analysis of cytokine secretion and to study its effects on isolated peripheral blood mononuclear cells (PBMCs, n=3).

Hierarchical clustering of RNAseq data identified 8 subclusters with similar regulatory patterns, with subcluster 3 genes downregulated and subclusters 4 and 5 genes upregulated by dexamethasone. Enrichment analysis showed that both up- and downregulated genes are involved in ossification and extracellular matrix organization pathways. The regulation of some of these genes aligns with the induction of osteogenesis, while other genes are regulated oppositely. Several pro- and anti-inflammatory genes were also differentially regulated, and qPCR analysis validated *CXCL1*, *CXCL8*, *IL18*, and *COL8A1* as unexpectedly upregulated, while *MMP1* and *CXCL12* expression was decreased by dexamethasone, as expected. Further in vitro experiments confirmed these results.

Secretion of IL-8 (product of *CXCL8* gene) was significantly increased by dexamethasone. However, PBMC responses indicated no significant pro- or anti-inflammatory effects of MSC conditioned medium.

In summary, dexamethasone dysregulated several genes associated with ossification, extracellular matrix organization, and inflammation. Considering the lack of effects of conditioned medium on PBMC responses, the role of pro-inflammatory mediators such as IL-8 in osteogenic differentiation and tissue remodelling remains to be clarified.

Po2_6.03

Targeting MCH Neuroendocrine Circuit in Lateral Hypothalamus to Protect Against Skeletal Senescence

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Abstract

Background: Neuroendocrine pathways are essential for metabolic homeostasis, yet their role in modulating bone metabolism and skeletal aging remains inadequately defined. Emerging evidence suggests that the brain may exert direct regulatory effects on bone health through neuroendocrine signaling. However, the specific neural circuits involved in osteogenesis and skeletal senescence have yet to be fully elucidated.

Methods: To explore this brain-bone connection, we utilized virus-based tracing to map neural circuits linking the lateral hypothalamus (LH), a key region governing energy metabolism, to bone tissue. Our focus was on melanin-concentrating hormone (MCH)-expressing neurons in the LH. Using chemogenetic tools, we selectively modulated the activity of these neurons in murine models to assess their impact on bone formation. Single-cell RNA sequencing was employed to investigate the influence of MCH signaling on the osteogenic differentiation of bone marrow stromal cells (BMSCs). Furthermore, micro-CT and histological analyses were conducted to evaluate bone mass and regeneration in age-induced osteoporosis and fracture healing models.

Results: We identified that MCH-expressing neurons in the LH are directly connected to bone tissue, and chemogenetic activation of these neurons enhanced osteogenesis, while their inhibition impaired bone formation. Notably, MCH was released into circulation upon activation of LH MCH neurons. Single-cell RNA sequencing revealed that MCH signaling facilitates osteogenic differentiation of BMSCs via MCHR1 and PKA pathways, and blocking MCH neuronal activity led to diminished osteogenesis and induced cellular senescence. In vivo, activation of LH MCH neurons significantly improved bone mass and promoted fracture healing in osteoporotic mice.

Conclusion: This study uncovers a previously unrecognized neuroendocrine circuit within the LH that governs bone metabolism and promotes osteogenesis. These findings provide new insights into the molecular mechanisms linking brain function to skeletal health and suggest that targeting MCH signaling may offer a novel therapeutic strategy for mitigating skeletal senescence and treating osteoporosis.

Keywords: MCH, neuroendocrine regulation, osteogenesis, BMSCs, skeletal senescence, osteoporosis.

Po2_6.04

Live Tracking of Cellular Functional Osteogenic Differentiation Using Spherical Nucleic Acid mRNA Nanoflare-based Sensors

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Abstract

Objectives Currently, there is an increasing focus on mesenchymal stromal cells (MSCs) as therapeutic option in bone pathologies and tissue engineering. The limited biological resources in bone regeneration make MSCs a promising cell population. Fluorescence sensors are becoming an efficient detection approach for many intracellular biomolecules. One exciting technology is the NanoFlare (NF)-based sensor, a type of spherical nucleic acid (SNA) technology, composed of gold nanoparticle (GNP) cores and densely packed and highly oriented oligonucleotide shells. Instant specific functional assessment of MSCs is critical for quality control in cellular therapy. Imaging of intracellular mRNAs not only helps us to better understand the formation and transcription of mRNAs, but also provides vital information for disease diagnosis and treatment. The aims of the current study are to design, synthesize and apply NF-based sensors on towards osteogenic differentiating MSCs and the cell line MG63 for live tracking expression of osteogenic early and late gene markers. **Methods** MSCs and the cell line MG63 were induced towards the osteogenic lineage for 3 and 7 days. Both cell types were incubated with specifically designed NF-based sensors to track their osteogenic gene marker expression. The fluorescence signal intensity was expected to specifically reflect intracellular mRNA expression during the osteogenic differentiation. The expression level of target mRNA was analyzed by quantifying the fluorescence value through live imaging and compared with RT-PCR. **Results** MSCs and the MG63 cell line showed solid mineralization when induced towards the osteogenic lineage compared to the controls. All synthesized NF-based sensors were successfully validated via sensitivity tests. Cell viability and cell proliferation of both cell types were not impaired when challenged with NF-based sensors. mRNA expression level in MSCs and MG63 induced towards osteogenic lineage showed many fold increase after quantification of fluorescence signals by live imaging of NF-based sensors compared to the respective control at day 3 or day 7. Moreover, mRNA expressions of the analyzed genes showed similar expression tendencies using RT-PCR. **Conclusions** Fluorescent NF-based sensors take advantage of the highly-efficient fluorescence quenching properties of GNPs, the effective cellular uptake of NF-based sensors that occurs without the use of any transfection agents, and the stability of such constructs to report the presence of intracellular target mRNA. The unique specific design of NF-based sensors enable the detection and live tracking of ultra-low concentrations of biomarkers in the complicated intracellular milieu. NF-based sensors represent a remarkable potential for future medical and biological applications.

Po2_6.05

Towards a 3D Organoid Model to study Effects of the Immune Checkpoints Programmed Cell Death Ligand 1 (PD-L1) and Its Receptor PD-1 on Bone Homeostasis

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Abstract

Objectives: Bone remodeling is maintained by the balanced action of osteoclasts and osteoblasts. The programmed cell death ligand 1 (PD-L1) and its receptor PD-1 provide inhibitory signals to regulate immune tolerance in immune-mediated diseases. However, their effects on bone health remains unknown. The aims of the current study were to investigate the bone architecture and osteoclast and osteoblast differentiation in PD-1 KO, PD-L1 KO and wild-type (WT) mice and to investigate the effects of corresponding recombinant proteins. Moreover, we analyzed the osteogenic differentiation in three-dimensional (3D) organoids from primary mouse MSCs and the osteoblastic cell lines MC-3T3-E1 and MG63. In this context we used spherical nucleic acid-based mRNA nanoflares (NFs) to track mRNA expression during osteoblast and osteoclast differentiation.

Method: Micro-computed tomography was used to evaluate the bone architecture. Osteoclast differentiation was induced by MCSF and RANKL and evaluated by TRAP staining and RT-PCR. Osteoclast functional activity was assessed by a bone resorption assay. MSCs were investigated for their vitality and proliferation. Additionally, MSCs were differentiated towards the osteogenic lineage. Mineralization was assessed by Alizarin Red S staining and extraction. 3D organoids were generated on agarose gel and analyzed using specific osteogenic stainings and RT-PCR. Cells were incubated with NFs and the signal intensity reflected target mRNA expression. The target mRNA expression level was analyzed by quantifying the fluorescence intensity values through live imaging and RT-PCR.

Results/Conclusion: In PD-L1 and PD-1 KO mice, bone density and trabecular structure were impaired. This effect is supported by increased osteoclastogenesis in both KO mice. MSCs derived from PD-1 KO mice showed a significant increase of mineralization compared to PD-L1 KO and WT mice. Interestingly, treatment of cells with recombinant PD-1 and PD-L1 (rPD-1, rPD-L1) showed significantly upregulated osteoblast-related gene expression in WT and PD-1 KO cells, whereas, PD-L1 KO cells indicated downregulation of most of the genes analyzed. WT and PD-L1 KO mice-derived MSCs showed decreased mineralization

after treatment with rPD-1 and rPD-L1, whereas those derived from PD-1 KO mice were increased. Moreover, most analyzed osteoclastogenesis gene markers were downregulated after treatment with rPD-1 and rPD-L1. Additionally, both primary MSCs and cell lines were able to self-organize into 3D organoids. Live tracking of mRNA expression level in towards osteogenic lineage induced cells showed many fold increase compared to the respective controls. Further research is needed towards establishing a 3D organoid model for an immune checkpoint therapy targeting bone homeostasis.

Po2_6.06

Optimizing MSC-based Therapies: Enrichment of Immunomodulatory Populations for Inflammatory Orthopedic Disorders

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Abstract

BACKGROUND: Mesenchymal stromal cells (MSCs) are of significant interest in translational research due to their immunomodulatory properties and relatively low risk of tumorigenesis. MSC-based therapies, using their immunomodulatory mechanism of action are already market approved for other inflammatory conditions. However, their application in inflammatory orthopedic disorders, including osteoarthritis, rheumatoid arthritis, and disc degeneration in chronic low back pain, remains to be explored. Despite this potential, long-term clinical trials with MSCs have shown variable efficacy in treating these conditions, possibly due to the heterogeneous nature of MSC populations [1].

OBJECTIVE: To identify surface markers for MSCs with the highest (hiMSCs) and lowest immunomodulatory potency (loMSCs) using functional immunomodulation assays.

METHODS: Three hundred-sixty surface marker on human bone marrow-derived MSCs (n=14 donors) were screened using a novel spectral-flow cytometry based screening methodology using fluorescent barcoding with anti-CD90/anti-CD73 to overcome batch effects (Figure 1A). MSC were isolated by plastic adherence and expanded to passage 4. Immunosuppressive capacity of the same MSCs was assessed by T cell suppression using a translationally relevant 3D co-culture (Figure 1B) of MSC spheroid with peripheral blood mononuclear cells (PBMCs) [2] MSC were primed with TNF- α and IFN- γ (20ng/ml each), formed into spheroids, and co-cultured with activated and CFSE stained PBMCs (n=3). The percentage of proliferating PBMCs was correlated with the MSC surface marker expression.

RESULTS: Two MSC preparations (#2 and #7) exhibited significant reduced T cell proliferation (Figure 1C), indicating a stronger immunosuppression. Out of the 360 markers, 8 showed a positive correlation ($R > 0.5$) and one a negative correlation (< -0.5). Three markers were consistently lower in the two MSC preparations with reduced T cell proliferation with one representative example shown in Figure 1D.

DISCUSSION: The identification of surface markers that correlate positively with immunosuppression allows to enrich 'untouched' hiMSC by removing loMSCs. Future studies will focus on validating this protocol in a proof-of-concept study, demonstrating the

enhanced immunosuppressive activity of hiMSCs compared to loMSCs. This novel surface-based selection strategy has great potential for optimizing MSC-based therapies and improving the treatment of inflammatory orthopedic diseases, such as osteoarthritis, rheumatoid arthritis, and Modic changes.

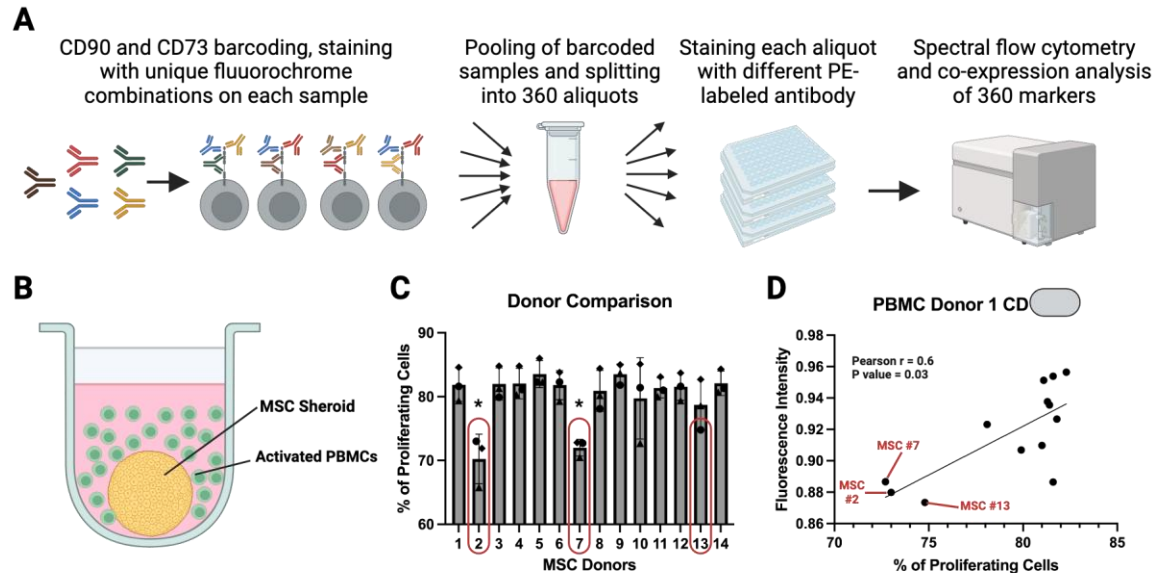


Figure 1: Identification of mesenchymal stromal cell (MSC) surface marker profile for different population of immunomodulation potential: A) Spectral flow cytometry-based surface marker screening, B) MSC-peripheral blood mononuclear cell (PBMC) 3D co-culture model, C) Percentage of proliferating PBMCs ($n=3$) suppressed by MSCs ($n=14$) (one-way ANOVA, $* = p < 0.05$) and D) Correlation between fluorescence intensity and percentage of proliferating PBMCs of MSCs ($n=14$) of 1 representative donor of 1 surface marker (blinded for IP protection).

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Po2_6.11

A Comparison of Bovine Adipose-Derived Mesenchymal Stromal Cells With Nucleus Pulposus Progenitor Cells: Exploring Extracellular Vesicle Therapy for Intervertebral Disk Regeneration

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Abstract

Introduction: Intervertebral disk (IVD) degeneration significantly contributes to low back pain (LBP), the leading cause of disability worldwide. While current therapies focus on conservative or surgical methods, their effectiveness remains debatable. Novel approaches focused on a nucleus pulposus progenitor cell population, positive for angiotensin receptor-1 (TIE2+), which showed regenerative and proliferative properties. Extracellular vesicles (EVs) play a key role in cellular communication and have gained considerable attention as potential cell-free treatments for LBP. Recent studies revealed that EVs isolated from nucleus pulposus cells (NPCs) differed from mesenchymal stromal cells (MSCs)-derived EVs with respect to enhanced cell proliferation in degenerated NPCs. Here, we compared the EVs secreted from three different bovine coccygeal sources: NPCs (TIE2+ and TIE2-) and donor-matched adipose tissue-derived MSCs (AdMSCs) with respect to number of vesicles, size distribution, membrane potential and proteomics.

Methods: NPCs and AdMSCs were isolated from 4 bovine tails obtained from a local abattoir. While MSCs were seeded directly, NPCs were sorted using fluorescence-activated cell sorting (FACS) to obtain TIE2+ and TIE2- populations. Cells were passaged once and cultured until 80% confluency. Standard medium was switched to EV-free medium for 2 days before harvesting for downstream proteomics and transcriptomics. EVs were isolated from conditioned media by ultracentrifugation and size exclusion chromatography. Additionally functional assays of the cells were performed: Colony forming unit (CFU) assays and bi-lineage differentiation (adipogenesis and osteogenesis) evaluated NPCs properties, while AdMSCs were tri-lineage differentiated (including chondrogenesis) (Fig.1).

Results: FACS revealed 0.1% to 12.3% TIE2⁺ cells among the NPCs. EVs were successfully isolated, yielding 1×10^9 (TIE2⁺), 0.8×10^9 (TIE2⁻), and 1.43×10^9 (AdMSC) particles per 0.5mL on average. Nanoparticle tracking analysis revealed purification of EV populations averaging 191, 193 and 176nm in size, with a mean zeta potential of -29.2, -27.6, -29.2mV, respectively. The CFU assay showed little differences between TIE2⁺ and TIE2⁻ NPCs. Differentiation assays revealed increased glycosaminoglycan production in AdMSCs during chondrogenesis, but adipo- and osteogenesis showed little differentiation for both NPCs and MSCs. Proteomic data confirmed differences between cells and EVs but showed absence of TIE2 protein, possibly due to detection limits of the shot-gun approach (Fig.2).

Conclusion: Here, EVs were successfully isolated from healthy donor-matched bovine NPCs and AdMSCs. Bovine-derived TIE2⁺ derived EVs was chosen as a pre-clinical model to investigate their regenerative potential compared to other targeted cell sources. The bovine source allows testing of the EVs regenerative effect in a bovine *ex vivo* IVD organ culture model.

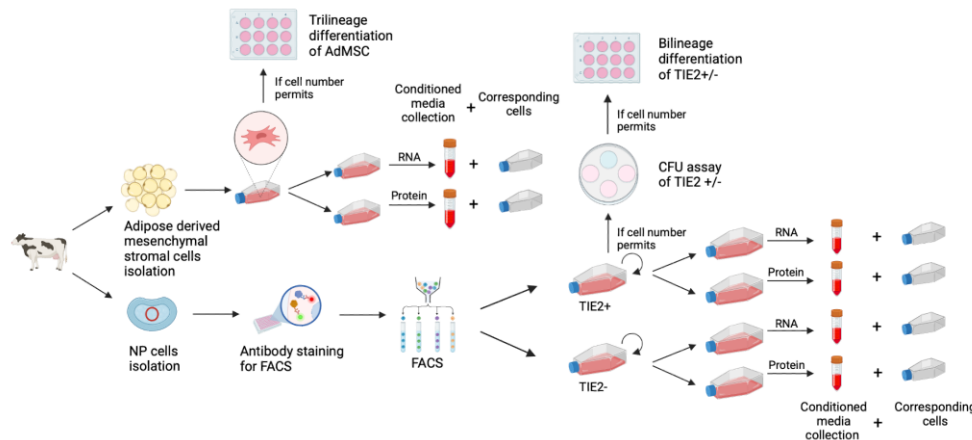


Figure 1: Graphical study design: Overview of the isolation of adipose-derived mesenchymal stromal cells (AdMSCs) and nucleus pulposus cells (NPCs) from bovine tissue, followed by fluorescence-activated cell sorting (FACS) for NPCs to obtain TIE2⁺ and TIE2⁻ cell populations. After expansion, cells and conditioned media (extracellular vesicles) were harvested for proteomics and transcriptomics analysis. Additionally, functional assays were performed: a colony forming unit (CFU) assay and a bi-lineage differentiation assay for NPCs, as well as a tri-lineage differentiation assay for AdMSCs. Figure created with Biorender.com

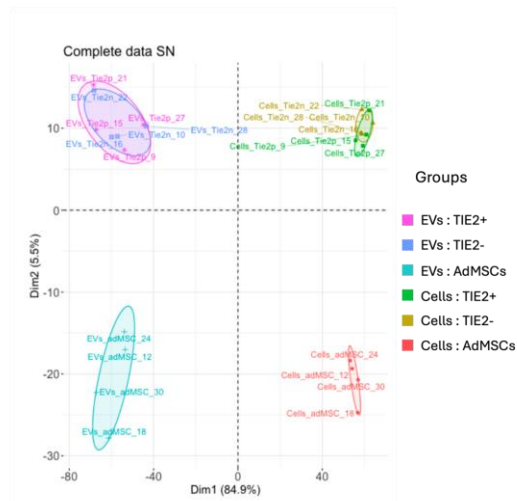


Figure 2: Principal component analysis of the log2-transformed Spectronaut intensity (SN), depicting three different cell types: TIE2+ and TIE2- nucleus pulposus cells, and adipose-derived mesenchymal stromal cells (AdMSCs), along with their corresponding extracellular vesicles (EVs).

Po2_6.13

Adipose stem cells from supra lata area : As efficient as abdominal adipose tissue source

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Abstract

Introduction: Adipose mesenchymal stem cells (AMSCs) are a promising source of multipotent cells for regenerative medicine. While AMSCs from abdominal adipose tissue have been well-investigated, those from the supra-fascia lata region remain largely underexplored, although the deep fat layer of the lateral thigh area is a prominent donor site in humans. This study compares the differentiation potential of AMSCs from these two anatomical regions in a porcine model. By investigation of their similarities and differences, this research may offer valuable insights into how anatomical origin influences regenerative potential. These insights could help determine the most suitable tissue source for regenerative applications such as bone regeneration and cartilage repair.

Materials and Methods: Adipose tissues were sterilely harvested from the abdominal and supra-fascia lata regions of Landrace pigs (n = 4). The tissues were minced and digested in 0.2% collagenase type 1-A at 37°C for one to two hours. The collagenase activity was stopped by adding the proliferation culture medium supplemented with fetal bovine serum, penicillin-streptomycin (1%), and amphotericin B (1%). The cells were washed, centrifuged at 1500 rpm, and resuspended in culture medium. After four passages, performed to enrich for proliferative populations, cells were characterized by immunohistofluorescence to identify the expression of CD105, CD90 and CD73, and then differentiated into osteogenic, adipogenic, and chondrogenic lineages.

Results: The immunohistofluorescence analysis confirmed the expression of mesenchymal stem cell markers (CD105, CD90 and CD73) at passage four for both cell sources, indicating

successful selection of stem cell. No significant differences were observed in the differentiation potential of the two sources into osteogenic, chondrogenic, and adipogenic lineages ($p > 0.05$). However, there was a slight superiority in the adipogenesis potential of the supra-fascia lata-derived cells, suggesting the presence of regional influences on differentiation.

Conclusion: This study provides valuable insights into the differentiation potential of AMSCs from two distinct anatomical regions. Although no statically significant differences were noted in their differentiation abilities, a subtle tendency towards variation in adipogenesis implies possible regional influences on lineage commitment. These findings especially lend credence to the feasibility of bone regeneration applications involving AMSCs derived from the abdominal and supra-fascia lata regions. Future research should also consider

additional factors such as age, sex, and lifestyle habits which could further refine the application of AMSCs in targeted tissue repair in humans.

Po2_6.14

Role of timing in mechanically induced *in vitro* cartilage formation

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Abstract

Osteoarthritis (OA) is a degenerative joint disease characterised by cartilage and surrounding tissues degradation and inflammation affecting 7.6% of the global population, particularly the elderly. OA leads to pain, stiffness, and loss of mobility and currently, there is no cure.

A regenerative treatment for AO is microfracture, where a focal cartilage lesion is debrided, and the subchondral bone plate is punctured multiple times to allow for mesenchymal stem cell (MSC) infiltration and new cartilage growth stimulation. However, the benefits are often temporary, as fibrocartilaginous tissue forms during healing. The postoperative rehabilitation protocol following microfracture plays a crucial, yet often overlooked, role in optimising long-term outcomes.

Despite advances in regenerative treatments, much of the underlying biology remains poorly understood. *In vitro* studies show that bone marrow-derived human MSCs can undergo chondrogenesis through mechanical loading alone, which activates endogenously produced latent TGF- β , a key factor in cartilage formation. Nonetheless, the impact of load timing on this process remains unexplored.

Timing is important because mechanical loading seems to be linked to the body's circadian rhythm – an intrinsic, 24-hour cycle that regulates most physiological processes. This rhythm is present in every cell, including MSCs and chondrocytes, where it maintains cartilage tissue homeostasis while its disruption is associated with OA progression. In cartilage, mechanical loading may reset circadian rhythms with the phase of the circadian cycle influencing the biological response to loading. These findings suggest that leveraging the circadian clock during rehabilitation could improve chondrogenesis and joint regeneration.

We hypothesise that optimising the timing of mechanical loading within the 24-hour day benefits cartilage repair. Ongoing *in vitro* experiments are being conducted using a multi-well, multiaxial load bioreactor. After monolayer expansion, human MSCs are seeded into gelatine methacrylate scaffolds and subjected to various loading regimes throughout the 24-hour cycle for two weeks in chondro-permissive medium. Afterwards, scaffolds are harvested and analysed for gene expression related to the circadian clock and chondrogenesis, as well as for glycosaminoglycan content in the scaffold and medium to assess cartilage matrix deposition. These analyses aim to gain a greater understanding of the role of timing of mechanical loading in rehabilitative chondrogenesis.

Po2_6.15

Stem Cell-Laden Hydrogels With Biomimetic Microenvironment for Osteochondral and Cartilage Tissue Engineering

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Abstract

Osteochondral damage from trauma or osteoarthritis is a general joint disease that can lead to an increased social and economic burden in the modern society. The inefficiency of osteochondral defects is mainly due to the absence of suitable tissue-engineered substrates promoting tissue regeneration and replacing damaged areas. The hydrogels are becoming a promising kind of biomaterials for tissue regeneration. The biomimetic hydrogel microenvironment can be tightly controlled by modulating a number of biophysical and biochemical properties, including matrix mechanics, degradation, microstructure, cell adhesion, and intercellular interactions. In particular, advances in stem cell-laden hydrogels have offered new ideas for the cell therapy and osteochondral repair. Herein, we report the importance of stem cell-laden hydrogels on promoting the development of osteochondral regeneration, especially in the field of manipulation of biomimetic microenvironment and utilization growth factors with various delivery methods.

Po2_7.02

The Biomechanical Properties of Hamstring Autografts Presoaked with Vancomycin Under Cyclic Loading

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Abstract

INTRODUCTION: Presoaking the graft with vancomycin before implantation has been shown to reduce the risk of postoperative infection after anterior cruciate ligament reconstruction (ACLR). However, the effects of presoaking on the graft biomechanical properties remain unclear. This study aimed to determine whether presoaking the graft with vancomycin affects the graft biomechanical properties and length after cyclic loading.

METHODS: Ten paired (20 specimens) gracilis and semitendinous tendons were harvested from fresh-frozen human cadaveric specimens. Two tendons were folded in half to make four strands, and the grafts were randomized into the vancomycin and control groups. The graft was exposed to the antibiotic solution for 15 min (5mg/mL), and prepared by mixing 1 g vancomycin with 200 mL normal saline (NaCl 0.9%). The control group was soaked in normal saline for 15 min. The prepared grafts were attached to the actuator of a dynamic tensile-testing machine. All grafts were tested with 3000 cycles of cyclic loading followed by a pull-to-failure (Fig. 1). The cyclic loading protocol consisted of position and load control blocks to simulate the graft in vivo in the postoperative phase after ACLR.

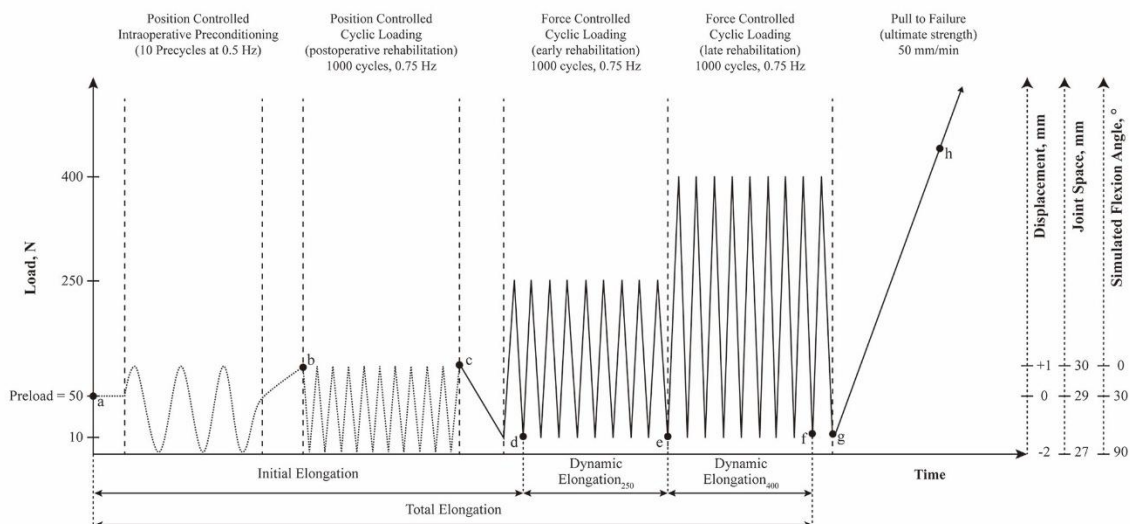


Fig. 1 The test protocol for the biomechanical testing with cyclic loading and a pull to failure.

RESULTS: Presoaking in vancomycin did not jeopardize the biomechanical properties of the graft. In addition, presoaking with vancomycin did not elongate the grafts. No significant differences were found in the mean Young's modulus and the mean total elongation of the graft of the specimen between the vancomycin group and the control group (Table 1).

Table 1. The biomechanical properties and the graft elongation according to the presoaking solution.

	Vancomycin (N = 10)	Control (N = 10)	P
Initial elongation [mm]	0.1 ± 0.1	0.1 ± 0.2	n.s.
Dynamic elongation [mm]	0.8 ± 0.1	0.9 ± 0.2	n.s.
Total elongation [mm]	0.9 ± 0.2	1.0 ± 0.3	n.s.
Young's modulus [GPa]	36.12 ± 4.7	35.11 ± 3.8	n.s.
Ultimate failure load [kN]	1.65 ± 0.53	1.67 ± 0.59	n.s.
Ultimate tensile Disp. [mm]	7.8 ± 2.1	8.1 ± 1.4	n.s.

DISCUSSION: Presoaking the graft with vancomycin jeopardized neither its biomechanical properties nor elongation even after cyclic loading in this in-vitro study. It is suggested that vancomycin presoaking could be considered a safe and effective preventive measure for postoperative infections after ACLR.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings suggest that presoaking hamstring grafts with vancomycin could be a safe and effective strategy to reduce the risk of postoperative infection in ACLR procedure.

Po2_7.05

Enhanced cell proliferation and cytoskeletal reorganization depending on cyclic stretch program of lapine Achilles tenocytes and human mesenchymal stem cells

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Abstract

Objective. Mobility can be severely impaired after tendon injuries. The self-healing and regenerative capacities of tendons are generally low, but might be increased by an adapted exercise concept. It is unclear whether HMSCs can also induce tenogenic differentiation in a very short time by cyclic stretching. However, the mechanoreponse of cell types contributing to tendon healing might differ. Therefore, the effect of two different protocols of cyclic stretching on both, lapine Achilles tenocytes (LATs) and human mesenchymal stem cells (HMSCs) after 2D stimulation will be investigated in detail.

Methods. For the 2D culture approach, silicone chambers (4 cm²) were seeded with LATs and HMSCs (6000 cells/cm²). After 24 hours of adherence, the chambers with adherent cells were exposed 8 days to either moderate or strenuous exercise (Different long cyclic stretch units, 4% + 0.3 Hz), while the control chambers remained unstimulated. At the end of each training session, viability was qualitatively assessed with a viability assay. DNA and sulfated glycosaminoglycan (sGAG) contents were quantitatively measured. Immunocytochemical staining of paxillin, F-actin, alpha smooth muscle actin and collagen type I was performed.

Results. The viability test showed that most of the LATs and hMSCs survived not only the moderate, but also the strenuous training. Cyclic stretching induced cytoskeleton reorganization in an elongated and more spindle like cell shape in comparison to unstimulated cells. The stronger cyclic stretching led to enhanced cell and F-actin stress fiber orientation against the stretch direction. Cell nuclei of both cell types became ellipsoid and more elongated due to the stimulation. Both cell types expressed paxillin, alpha smooth muscle actin and collagen type I. The expression was upregulated by cyclic stretching, especially by the strenuous exercise. Quantitative analyses confirmed that moderate stretching had a greater induction on the sGAG synthesis and proliferation of hMSCs than on LATs. After stretching, HMSC align at an angle of 90-100° and LAT (100-120°).

Conclusions. The differences in cell viability of LATs compared to hMSCs and their dependence on the stretching program as reported by others could be confirmed in the present study. As expected, the mechanoreponse to strenuous exercise exceeded that to moderate stimulation in both cell types. Although cell reactions of LATs and HMSCs were mostly similar some aspects differed.

Acknowledgements. The work is financially supported by the Nürnberger Versicherung and the Kerscher Diabetes Foundation.

Keywords. Cyclic stretch, Achilles tenocytes, human mesenchymal stem cells, tenogenesis,

Po2_7.06

Comparative efficacy and safety of different surgical approaches for the treatment of anterior cruciate ligament injury: a Bayesian network meta-analysis

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Abstract

Purpose. To compare the efficacy and complication rates of different approaches for the treatment of anterior cruciate ligament (ACL) injury.

Methods. A systematic literature search was conducted to identify randomized controlled trials (RCTs) published from database inception to June 1, 2024, comparing different therapeutic approaches for the treatment of ACL injury, including primary repair, primary repair with augmentation (PRA), ACL reconstruction (ACLR) with remnant preserving (ACLR+RP), ACLR with remnant debridement (ACLR+RD), ACLR with augmentation (ACLRA), rehabilitation and delayed ACLR (Reha+ACLR) and rehabilitation only. The primary outcomes were knee stability and functional recovery at 24 months and last follow-up post-surgery. Secondary outcomes included the rate of revision, and complication rates. Summary standardized mean differences (SMDs) or odds ratios with 95% credible intervals (95% CrIs) were estimated using Bayesian network meta-analysis with random effects.

Results In total, 17 RCTs comparing 1404 patients were included. Preoperative knee function scores showed no significant differences between groups, and there were no significant differences in gender, age or the time from injury to surgery. At the 24-month follow-up, the ACLR+RP group demonstrated superior functional recovery scores compared to the ACLR+RD group (SMD=-0.45, 95%CrI: -0.83 to -0.06). The final follow-up results indicated that ACLR+RP outperformed the ACLR+RD (SMD=-0.51, 95%CrI: -0.81 to -0.19), Reha+ACLR (SMD=-0.57, 95%CrI: -1.15 to -0.004) and rehabilitation (SMD=-0.59, 95%CrI: -1.05 to -0.06) groups. No significant differences were observed among the other groups. The difference in patient functional scores from baseline to the end of follow-up was significantly better in the ACLR+RP group compared to the ACLR+RD group (SMD=-3.00, 95%CrI: -6.08 to -0.05). Regarding revision rates and postoperative complications, there were no significant differences between the groups.

Conclusions The study suggests that ACL reconstruction with remnant

preserving provides superior functional recovery compared to other surgical and rehabilitation approaches without increasing revision or complication rates.

Table 1. Detailed results of network meta-analysis for joint function at 24-months (grey) and the last-follow up (white).^{a,c}

Primary repair ^b							
	0.45 (-0.11, 1.04) ^c	0.78 (0.10, 1.50)^c	0.27 (-0.35, 0.92) ^c	0.12 (-0.83, 1.11) ^c	0.04 (-0.61, 0.72) ^c	0.15 (-0.52, 0.89) ^c	
0.53 (-0.14, 1.21) ^c	PRA^b	0.33 (-0.12, 0.79) ^c	-0.17 (-0.52, 0.16) ^c	-0.32 (-1.12, 0.46) ^c	-0.41 (-0.84, 0.01) ^c	-0.30 (-0.73, 0.19) ^c	
0.85 (-0.04, 1.74) ^c	0.32 (-0.35, 0.98) ^c	ACLR+RP^b	-0.51 (-0.81, -0.20)^c	-0.66 (-1.43, 0.12) ^c	-0.74 (-1.20, -0.29)^c	-0.63 (-1.08, -0.12)^c	
0.40 (-0.40, 1.20) ^c	-0.13 (-0.66, 0.42) ^c	-0.45 (-0.83, -0.06)^c	ACLR+RD^b	-0.15 (-0.86, 0.56) ^c	-0.23 (-0.59, 0.10) ^c	-0.13 (-0.46, 0.28) ^c	
0.86 (-0.41, 2.08) ^c	0.32 (-0.79, 1.42) ^c	-0.0007 (-1.02, 1.01) ^c	0.45 (-0.49, 1.39) ^c	ACLRA^b	-0.08 (-0.88, 0.71) ^c	0.03 (-0.74, 0.87) ^c	
0.10 (-0.78, 0.97) ^c	-0.43 (-1.15, 0.28) ^c	-0.75 (-1.57, 0.04) ^c	-0.30 (-1.01, 0.41) ^c	-0.76 (-1.93, 0.46) ^c	Reh+ACLR^b	0.10 (-0.3, 0.59) ^c	
0.48 (-0.33, 1.28) ^c	-0.06 (-0.64, 0.57) ^c	-0.37 (-1.02, 0.29) ^c	0.08 (-0.45, 0.62) ^c	-0.37 (-1.47, 0.74) ^c	0.38 (-0.28, 1.05) ^c	Rehabilitation^b	

The positive value means knee function score recovery in favor of the second intervention, whereas the negative value means knee function score recovery in favor of the first intervention for each comparison. SMD (95% CI). PRA, primary repair with augmentation; ACLR, anterior cruciate ligament reconstruction; RP, remnant preserving; RD, remnant debridement; Reh, rehabilitation.^{a,c}

Po2_7.07

Biomimetic Poly- ϵ -caprolactone Braids for Anterior Cruciate Ligament Reconstruction: An Analysis of the Influence of the Filament Arrangement on Mechanical Behavior

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Abstract

Purpose: This study investigates the influence of filament arrangement on the mechanical performance of braided poly- ϵ -caprolactone (PCL) scaffolds as potential Anterior Cruciate Ligament (ACL) implants as a tissue engineering approach.

Introduction: ACL ruptures are still commonly treated by using autologous implants. Due to their limited availability and the weakening of the secondary knee stability, braided degradable scaffolds have been engineered as synthetic solution for ligament reconstruction. These scaffolds provide structural support and promote tissue remodeling through cellular ingrowth. [1, 2] As further approach the arrangement of the filaments is varied to compare the mechanical behavior of different biomimetic braiding structures based on the bundled structure of ligaments. [3, 4]

Methods: Braided PCL scaffolds were fabricated from melt-spun PCL monofilaments. [5, 6] The filament arrangement was varied into three designs with constant number of filaments. These can be differentiated into one layer-based and two bundle-based and were all conceived to replicate a native ACL. For the layer-based design static tests confirm that their stiffness, tensile strength and degradation behavior match those of a native ACL. [7] All designs are tested in load to failure tests with a preload of 30 N, standardized 40 mm gauge length and testing speed of 40 mm/min.

Results: The tests show that the filament arrangement has an impact on the maximal tensile force, the way of rupture and the diameter of the scaffold. The layer-based design reaches a maximal tensile force of 4113 ± 187 N, is nearly ruptured apart and has a tension-free diameter of 9 mm. [7] The bundle-based designs differ in their mechanical performance. One design results in a maximal tensile force of 4000 N with a complete rupture and a tension-free diameter of 7 mm. The other bundle-based design has a maximal tensile force of 3000 N with no rupture of the inner braids and a tension-free diameter of 8 mm.

Conclusion: The tests show that the filament arrangement impacts the mechanical behavior of braided scaffolds. All designs can ensure a minimum tensile strength of 2000 N as a native ACL and higher. [7] It is also shown that by a different filament arrangement the outer diameter of the scaffold can be decreased. A smaller diameter could bring advantages for the surgical implantation. Further mechanical parameters such as the stiffness must be analyzed. Also the testing of cell ingrowth is of special interest due to the biomimetic structure of the bundle-based design.

Po2_7.08

Age-Related Alterations in 3D Microvasculature of Equine Tendons: Insights from Immunolabelling and Micro-Computed Tomography

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Abstract

Introduction: Age-related tendon injuries are prevalent in humans and horses, with limited treatment options due to a poor understanding of the mechanisms driving age-related dysfunction. Vascular cells localize to the tendon interfascicular matrix (IFM) and become dysfunctional with age, but tendon vasculature and age-related changes remain poorly defined. This study investigates 3D microvascular structural and molecular changes in equine tendons with ageing using 3D immunolabelling and contrast-enhanced micro-computed tomography (μ CT).

Methods: Forelimb superficial digital flexor tendons (SDFTs) were harvested from young (2-5 years, $n=4$) and old (>17 years, $n=4$) horses euthanised at a commercial abattoir for reasons other than tendon injury. Immunohistochemistry was performed on longitudinal SDFT cryosections using established vascular cell markers. SDFT pieces (5x5x2mm) were fixed and cleared prior to 3D immunolabelling with the vascular marker VWF and confocal imaging (4.6 μ m pixel size). For μ CT imaging, equine distal forelimbs were perfused with barium sulphate gelatin solution, the SDFT was harvested, cut into 15mm-length pieces, and fixed prior to imaging (10 μ m voxel size). Images were analysed using ImageJ, Avizo and CTAn. Differences ($p<0.05$) between age groups were determined using T-tests (GraphPad Prism).

Results: Immunolabelling showed several endothelial cell, pericyte and smooth muscle cell markers in tendon microvessels which localised to the IFM (Fig. 1a). Analysis of 3D VWF-immunolabelled images showed significantly higher VWF volume in tendon microvasculature with ageing (Fig. 1b, $p=0.0373$). μ CT images (Fig. 2a) were analysed to calculate the full-volume tendon vasculature; young tendons had significantly greater vessel volume (Fig. 2b, $p=0.0018$) and vessel diameter (Fig. 2c, $p=0.0145$) than old tendons.

Discussion/Conclusion: This study provides the first comprehensive characterisation of the tendon 3D microvasculature. μ CT imaging demonstrated an extensive vascular network in the IFM, while immunolabelling revealed its capillary network. μ CT analysis showed a significant decrease in vascular density and diameter with ageing, whereas immunolabeling suggested an increase in VWF-labelled volume with ageing, suggesting an expansion in capillary density not apparent in the μ CT images. These dysregulations may contribute to the age-related decline in tendon function and increased risk of injury.

Funder: Horserace Betting Levy Board (prj/804).

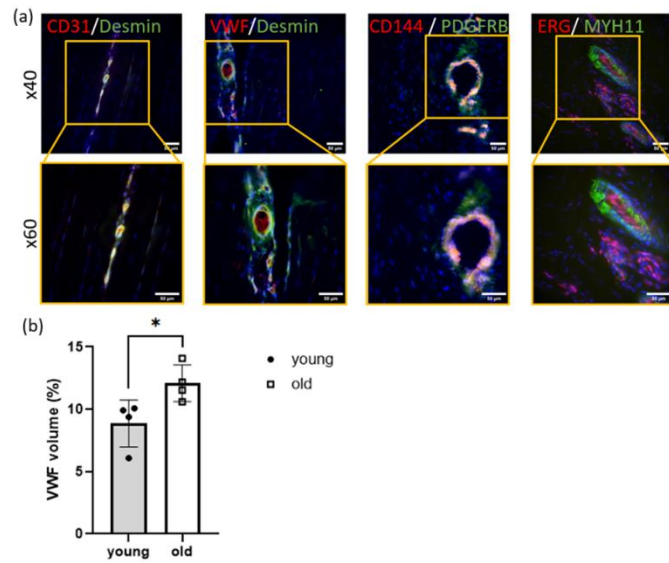


Figure 1. (a) Representative images of tendon microvasculature. Red; endothelial markers, green; mural cell markers, blue; nuclei. (b) VWF-labelled volume in young and old tendons. * $p < 0.05$.

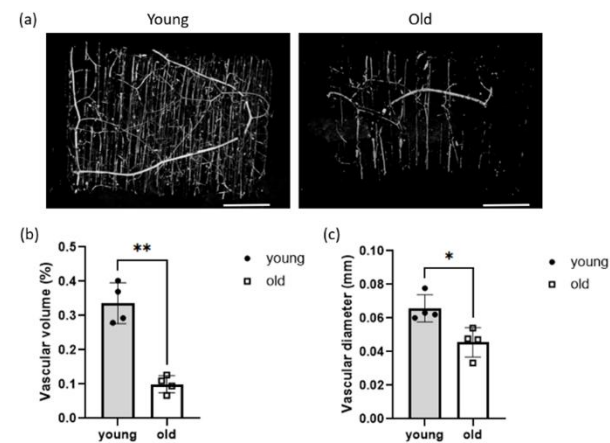


Figure 2. (a) Representative 3D reconstructed μ CT images of young and old tendon microvasculature. Scale bar is 5mm. (b) Vascular volume and (c) vascular diameter in young and old tendons. * $p < 0.05$ ** $p < 0.01$.

Po2_7.09

Analysis of imaging presentations, full endoscopic evaluation, and pathological characteristics of a novel interlaminar ligament.

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Abstract

Background: This study aims to analyze the characteristics of an unnamed interlaminar ligament (ILL) through magnetic resonance imaging (MRI), endoscopy, and pathological examination.

Method: A retrospective study was conducted to review the clinical data of patients who underwent posterior endoscopic surgery for lumbar disc herniation or lumbar spinal stenosis at our medical center between January 2021 and February 2022. The height, width, and cross-sectional thickness of the ligament were measured using T2-weighted MRI. Additionally, the morphological and pathological characteristics of the ILL were compared with those of the ligamentum flavum (LF) to highlight structural differences between the two ligaments.

Results: Forty-three patients were included in this study, comprising 27 males and 16 females, with a mean age of 46.6 ± 12.1 years. There were 20 cases of lumbar disc herniation and 23 cases of lumbar spinal stenosis. In the lumbar disc group, the width, length, and thickness of the ILL were 17.7 ± 3.5 mm, 4.3 ± 1.3 mm, and 18.3 ± 3.5 mm, respectively. The corresponding parameters for the LF were 5.3 ± 1.9 mm. In the lumbar spinal stenosis group, the width, length, and thickness of the ILL were 16.0 ± 3.1 mm, 4.1 ± 1.6 mm, and 17.6 ± 4.8 mm, respectively, and the LF thickness was 6.3 ± 0.8 mm. The difference in LF thickness between the two groups was statistically significant ($P = 0.02$). Surgical time also differed between the two groups ($P = 0.04$). Endoscopic analysis revealed differences in the location of the anchor points and appearance of the ligaments. Additionally, biopsy results showed significant differences in the density and direction of fibrous structures between the ILL and LF. Endoscopy also revealed a significant difference in the ILL thickness grades when compared across disease types ($P = 0.09$).

Conclusion: The interlaminar ligament is a previously unnamed structure with significant structural differences compared to the ligamentum flavum. Clinically, it serves as the endpoint for soft tissue channel creation during posterior endoscopic procedures. MRI thickness of the LF and endoscopic thickness of the ILL vary according to the disease spectrum.

Po2_7.10

The ageing tendon extracellular matrix is compromised by senescent cells.

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Abstract

Tendons are frequently injured, not only in athletes, but also during normal everyday activities, particularly as we get older. Tendon injuries are characterised by a lack of treatments, poor recovery, and high rates of reinjury; and increasingly become a limiting factor to quality of life in our ageing population. Horses also often suffer from tendon injuries, and provide an excellent model in which to study tendon ageing. The equine superficial digital flexor tendon (SDFT) is a well-established model of the human Achilles, with homologous energy-storing function and structure, and subject to aetiologically similar tendinopathies.

Here we characterise links between cellular senescence and age-associated tendon dysfunction. Beta-galactosidase staining demonstrates senescence in cells isolated from aged SDFTs, and cells from aged tendons (13-19 years) exhibit significantly slower growth rates than from young tendons (3-6 years, Figure 1). By inducing replicative senescence in cells isolated from the SDFTs of young horses, confirmed by markers of growth arrest, and performing bulk RNA sequencing we identify detrimental characteristics of ageing in tendon which are driven at the cellular level. In addition to upregulation of canonical senescence markers, we find that senescence in tenocytes particularly affects the components of the tendon extracellular matrix (Figure 2). Transcripts corresponding to collagens, proteoglycans, and elastic fibres - which make up the majority of the tendon tissue - are drastically dysregulated in senescent tenocytes, along with the matrix metalloproteinases which regulate these protein groups.

Some proteins within the tendon have been shown to have exceptionally long half-lives, and a delicate matrix composition/organisation is thought to underlie the poor reparative capacity of tendon tissue. Together, these results suggest a senescence-associated secretory phenotype specific to aging tenocytes, which likely compromises both the synthesis and turnover of proteins crucial to energy-storing tendon function.

Funded by the Biotechnology and Biological Sciences Research Council (BB/W007282/1).

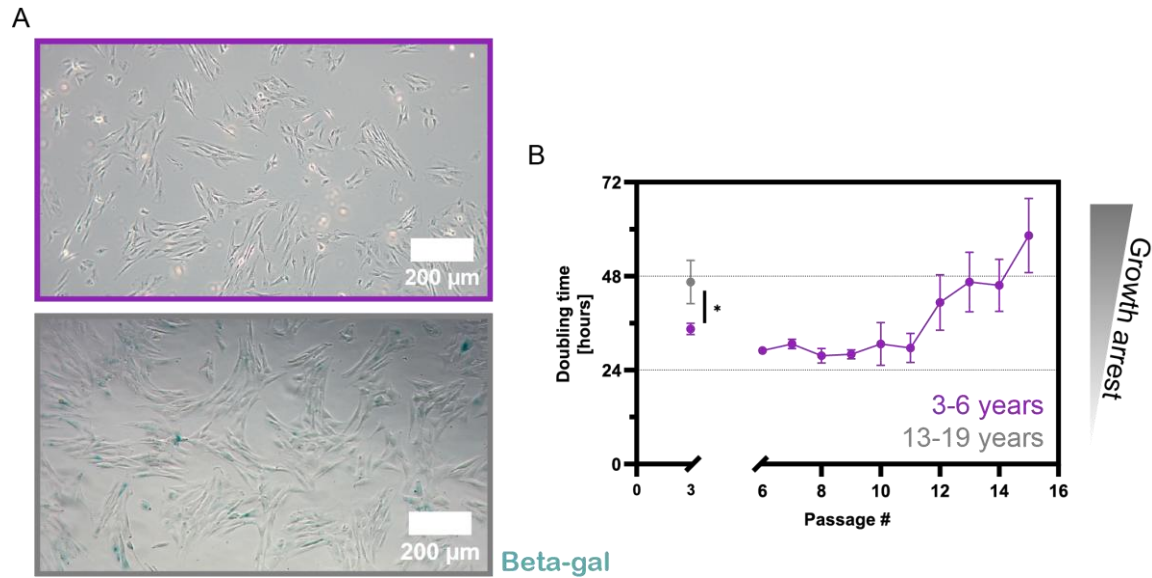


Figure 1: Repeated passaging induces senescence in equine tenocytes and reflects in vivo tendon ageing.

(A) Beta-galactosidase staining of equine tenocytes isolated from 4-year (purple) and 19-year (grey) SDFTs. (B) In vitro doubling times of equine tenocytes (N≥3).

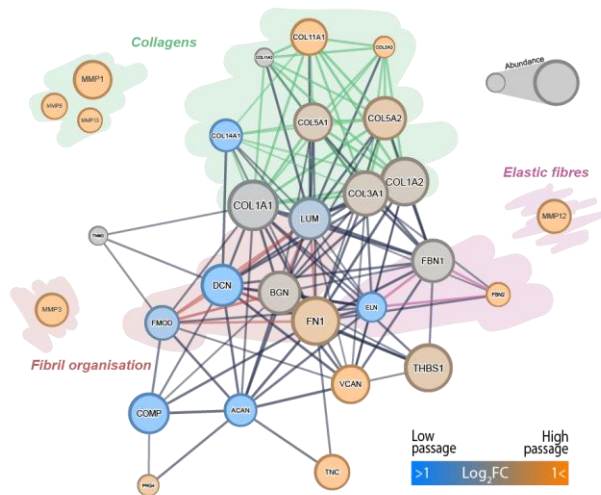


Figure 2: Sequencing identifies dysregulation of the tendon extracellular matrix by senescent cells.

Network of proteins (nodes) with known function in the tendon extracellular matrix and interactions (edges) between them. Matrix metalloproteinases specific to proteins within the network are also shown. Node size and colour reflect its abundance in the tenocyte transcriptome and fold-change from low- to high-passage (N=4), respectively.

Po2_7.12

The Cambridge Knee Injury Tool (CamKIT): A Clinical Prediction Tool for Acute Soft Tissue Knee Injuries

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Abstract

Soft tissue knee injuries (STKIs) significantly burden healthcare systems. Despite advances in prevention and rehabilitation, rising injury rates and increasing reliance on surgical interventions highlight the urgent need to optimise diagnostic and management pathways for STKIs.

The Cambridge Knee Injury Tool (CamKIT) is an innovative 12-point clinical prediction tool incorporating patient factors, external factors, and clinical signs and symptoms. It was validated retrospectively on 229 patients presenting with acute knee pain to a major Regional Trauma Center over three months. Data on the twelve variables, injury outcomes, and treatment pathways were extracted from individual Electronic Medical Records (EMR). The study evaluated the tool's potential to improve time-to-event for consultations, MRI, and surgery and assessed resource allocation in discharge, consultation, and MRI utilisation.

Among 229 patients, 70 clinically significant injury events were identified, with 44% (31/70) occurring in isolation and 56% (39/70) being concomitant. ACL injuries were most common, accounting for 54% (38/70) of all events, with 90% (34/38) being concomitant. Collateral ligament injuries were also prevalent, with 33% (23/70) involving the MCL and 16% (11/70) the LCL, all of which were concomitant. Meniscal injuries were recorded in 39% (27/70) of cases involving the medial meniscus and 21% (15/70) involving the lateral meniscus.

Only 30% (70/229) of patients presenting to A&E had confirmed injuries, and 17% (39/229) required surgery. Among 116 referred for specialist consultation, 59% (68/116) had confirmed injuries, with 34% (39/116) requiring surgery. MRI was performed in 37% (84/229) of cases, with 76% (64/84) showing confirmed injuries and 46% (39/84) requiring surgery. The CamKIT demonstrated a median score of 7.5 (IQR = 3) in the injured cohort versus 2 (IQR = 3) in the non-injured cohort, showing a statistically significant difference ($p < 0.0001$).

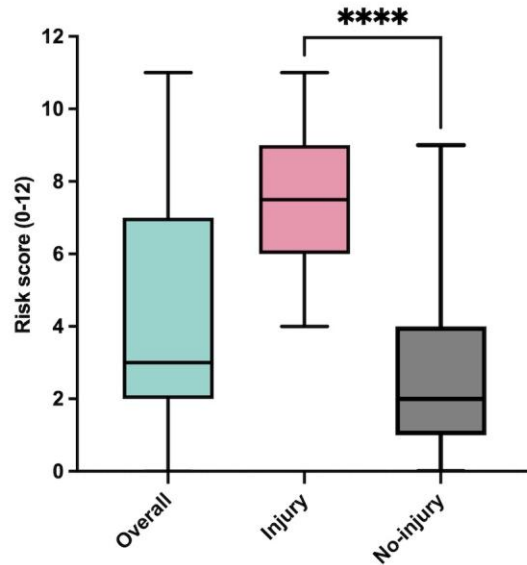


Figure 1. CamKIT risk scores for overall, injured, and non-injured cohorts.

Constructed as a three-tier risk stratification tool, CamKIT achieved 100% sensitivity, 94.3% specificity, an 89% positive predictive value, and 100% negative predictive value. It expedited imaging for 70% of patients, accelerated surgery pathways for 85%, and reduced specialist consultations by 34% without missing any clinically significant injuries.

The findings underscore the inefficiencies in current referral pathways, with a low proportion of significant injuries among A&E presentations. CamKIT addresses these challenges by enabling accurate, accessible diagnostics in acute settings, empowering primary care clinicians, improving secondary care efficiency, and potentially reducing patient wait times and healthcare costs.

Po2_7.13

Assessing Diagnostic Challenges in Acute Soft-tissue Knee Injuries: A Delphi Study

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Abstract

Soft tissue knee injuries (STKIs) are among the most prevalent musculoskeletal injuries, often presenting in acute settings. Accurate assessment and effective management require a comprehensive history and physical examination. However, frequent misdiagnoses highlight significant challenges within the diagnostic pathway. This modified e-Delphi study aimed to address these challenges by exploring healthcare stakeholders' perceptions of the importance of patient and external risk factors, clinical signs and symptoms, special clinical tests, and diagnostic imaging in diagnosing STKIs.

The study was conducted over three rounds with 32 orthopaedic healthcare stakeholders, including physiotherapists, emergency nurse practitioners, sports medicine physicians, radiologists, orthopaedic registrars, and consultants. Using a structured process, participants scored and ranked various diagnostic components on a 10-point Likert scale. Feedback was used to refine items progressively, leading to consensus on the priorities for diagnosing STKIs.

The first round revealed significant variability in stakeholders' opinions, underscoring the subjective and complex nature of diagnosing STKIs. By the second round, patterns emerged regarding the perceived importance of specific diagnostic items. In the final round, participants reached a consensus, producing refined rankings of the diagnostic components.

The results underscored the lack of accurate and readily available diagnostic methods in acute care, posing barriers to timely and effective diagnosis and potentially leading to suboptimal outcomes. Participants consistently emphasised the need for a patient-centered approach, considering individual circumstances and preferences to enhance diagnostic accuracy and management plans. The study identified the potential for a paradigm shift in diagnosing acute STKIs. Establishing a uniform, evidence-based framework could address the variability in diagnostic approaches, offering clear guidelines and standardised procedures. Such a framework would improve diagnostic accuracy, optimise resource allocation, and enhance patient outcomes. Additionally, the findings highlighted the need for more accessible and cost-effective diagnostic tools in acute settings. These tools could support the implementation of standardised diagnostic frameworks, ensuring consistency across diverse clinical environments.

In conclusion, this modified e-Delphi study highlights the complexities and challenges of diagnosing STKIs in acute care. It underscores the importance of a standardised, patient-centered, and evidence-based diagnostic framework. By addressing these challenges, healthcare providers can improve the accuracy of STKI diagnoses, reduce variability in care, and ultimately enhance outcomes for patients with these common musculoskeletal injuries.

Po2_7.14

PIEZO1 promotes tendon growth in adults via mechanical stress

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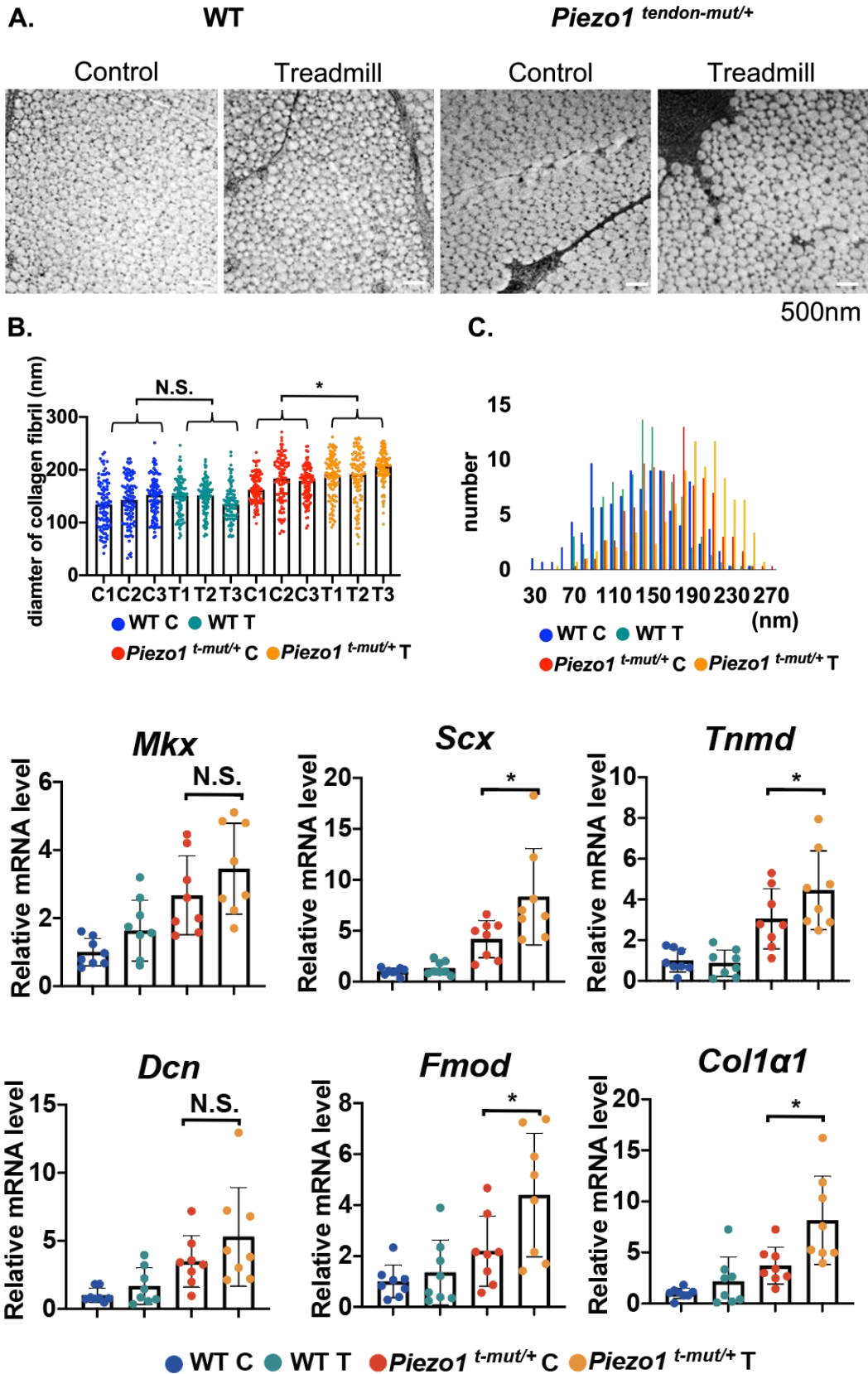
Abstract

Introduction: PIEZO1 is a mechanosensor implicated in tendon function, but the specific mechanical stresses it senses *in vivo* remain unclear. Previous studies demonstrated that PIEZO1 activation combined with tensile stress upregulates MKX and SCX expression, critical transcription factors for tendon development and homeostasis. Additionally, appropriate mechanical stress is known to promote anabolic effects in tendon tissue. We hypothesized that PIEZO1 senses running-induced stimulation *in vivo*, enhancing tendon anabolic processes and that PIEZO1 activation amplifies these effects.

Methods: To investigate the role of PIEZO1 *in vivo*, 14-week-old *Scx-cre: Piezo1^{cx/+}* mice (tendon-specific gain-of-function PIEZO1 mice, Piezo1 t-GOF) and *Piezo1^{cx/+}* control mice (WT) were subjected to treadmill exercise. Mice ran at 12 m/min on a flat incline for 1 hour daily over 4 weeks. Achilles tendons were collected post-exercise for morphological analysis via electron microscopy and RNA expression analysis via qPCR. Collagen fibril diameter (100 fibrils per sample) was measured from cross-sections, and tendon-related gene expression (e.g., *Mkx* and *Scx*) was quantified. Statistical significance was assessed using the Student's t-test and Tukey's multiple comparison test.

Results: Electron microscopy revealed increased collagen fibril diameter in Piezo1 t-GOF mice after treadmill exercise. Mean fibril diameters were as follows: WT without exercise, 140.6 nm; WT with exercise, 135.0 nm; Piezo1 t-GOF without exercise, 172.3 nm; and Piezo1 t-GOF with exercise, 190.4 nm (N=3 per group) (Figure 1). qPCR analysis showed upregulation of *Mkx* and *Scx* in Piezo1 t-GOF mice post-exercise (N=8 per group) (Figure 2).

Discussion: PIEZO1 activation enhances tendon anabolic responses to mechanical stress, promoting increased collagen fibril diameter and upregulation of key tendon-related genes, SCX and MKX. These transcription factors are essential for tendon homeostasis and decline with age, contributing to tendon degeneration. Our findings suggest that PIEZO1 activation may counteract these degenerative changes, improving tendon structure and function. This highlights PIEZO1's potential as a therapeutic target to maintain tendon homeostasis, potentially preserving physical performance and quality of life.



Po2_7.17

The effect of mechanical stress on enthesis homeostasis in a rat Achilles enthesis organ culture model

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Abstract

This study aimed to elucidate the effects of mechanical stress on the structure and homeostasis of the enthesis using an ex vivo rat Achilles enthesis organ culture model. The model included the Achilles tendon and calcaneus, subjected to three conditions: 1-mm cyclic tensile strain (CTS), 2-mm CTS (both at 0.5 Hz, 4 hours daily for one week), and no stress. Histological analysis, transmission electron microscopy (TEM), live/dead staining, and molecular evaluations were conducted to assess structural integrity, cell viability, and gene expression.

The 1-mm CTS model effectively preserved the structure of the enthesis, maintaining periosteal fibrocartilage and collagen fiber alignment. In contrast, the no-stress model exhibited fibrocartilage disappearance and disorganized collagen fibers. The 2-mm CTS model displayed disrupted transitional zones, fibrocartilage mineralization, and a distinct tide mark separating unmineralized and mineralized fibrocartilage. TEM revealed poorly defined borders between fibrocartilage and mineralized fibrocartilage in the no-stress model and significant mineralization in the 2-mm CTS model.

Live/dead staining demonstrated that cell viability was highest in the 1-mm CTS group, indicating reduced apoptosis under mild mechanical stress. Gene expression analysis showed increased levels of scleraxis (Scx) and sex determining region Y-box 9 (Sox9) in the 1-mm CTS group, markers associated with tendon differentiation and chondrogenesis, respectively. Conversely, the 2-mm CTS model upregulated Runt-related transcription factor 2 (Runx2), associated with osteogenesis, and matrix metalloproteinase 13 (Mmp13), linked to matrix degradation and degeneration.

These findings highlight the critical role of mild mechanical stress (1-mm CTS) in maintaining the homeostasis and structural integrity of the enthesis, supporting fibrocartilage and collagen preservation, and promoting cell survival. Conversely, excessive stress (2-mm CTS) induces degenerative changes, including fibrocartilage mineralization, collagen fragmentation, and increased expression of markers associated with ossification and matrix breakdown. This study underscores the importance of controlled mechanical stress in enthesis health and has implications for understanding enthesis pathology and its potential therapeutic interventions in orthopedic sports medicine.

Figure 1 Histological sections at the enthesis with Toluidin Blue and Picro Sirius Red staining

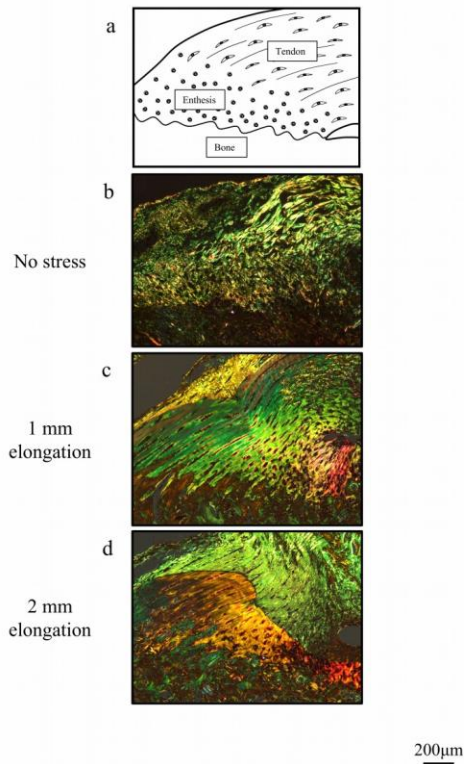
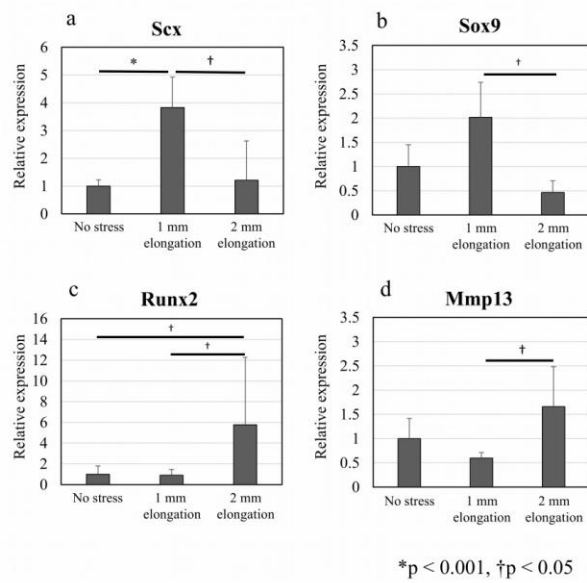


Figure 2 The effects of mechanical stress on mRNA expression of Scx, Sox9, Runx2, and Mmp13 at the enthesis



Po2_7.19

Anterior cruciate ligament in the osteoarthritic human knee: fibril-level changes revealed by the atomic force microscopy

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Abstract

Knee osteoarthritis (OA) is a disease of the whole joint, and concomitantly with changes in cartilage and bone, changes occur in the anterior cruciate ligament (ACL), including dispersion and degradation of the collagen fibre network at the micrometer scale [1]. However, it is unknown whether changes occur in the collagen fibrils of ACLs at the nanometer scale. Therefore, this study aimed to characterize the D-period and diameter of collagen fibrils from anteromedial (AM) and posterolateral (PL) bundles of human ACLs and relate these to the stage of patellofemoral joint OA.

Fascicles were extracted from AM and PL bundles of 18 cadaveric human ACLs (Fig. 1a) and mounted onto glass slides in air. Atomic force microscopy (AFM) imaging was performed within 2–4 hours in non-contact mode using a 2×2 µm scan area with a resolution of 512×512 pixels, and a scan rate of 1 Hz, to measure the nanometer-scale surface topography. 30 fibrils per sample were analyzed to determine the average and standard deviation of diameter and D-period length (Fig. 1b). The stage of OA was characterized by OARSI-grading of cartilage at the patella, femoral groove, anterior and posterior lateral, and medial femur. The relationships between the fibril diameter, D-period length, and OARSI-grades were investigated with Spearman's rank correlation, individually for the bundles.

In the AM bundle, the fibril diameter ($\rho=0.597$, $p=0.009$, Fig. 2a) and its standard deviation ($\rho=0.506$, $p=0.032$, Fig. 2b) positively correlated with femoral groove OARSI grade. Additionally, the standard deviation of D-period negatively correlated with patellar cartilage OARSI grade ($\rho=-0.503$, $p=0.033$). In the PL bundle, the fibril morphological parameters did not show correlation with OARSI grades.

The results demonstrated that the AM bundle fibril diameters increased and their diameter distribution broadened at progressive stages of femoral groove OA, while the D-periods became more uniform with patellar OA. These findings may indicate molecular rearrangement or reduced synthesis of thinner collagen fibrils within the AM bundle. Further studies using advanced imaging and molecular techniques (e.g., immunohistochemistry and confocal microscopy) are needed to explore these mechanisms.

Reference: [1] Hasegawa et al. (2012), *Arthritis Rheumatol*, 64(3), 696-704

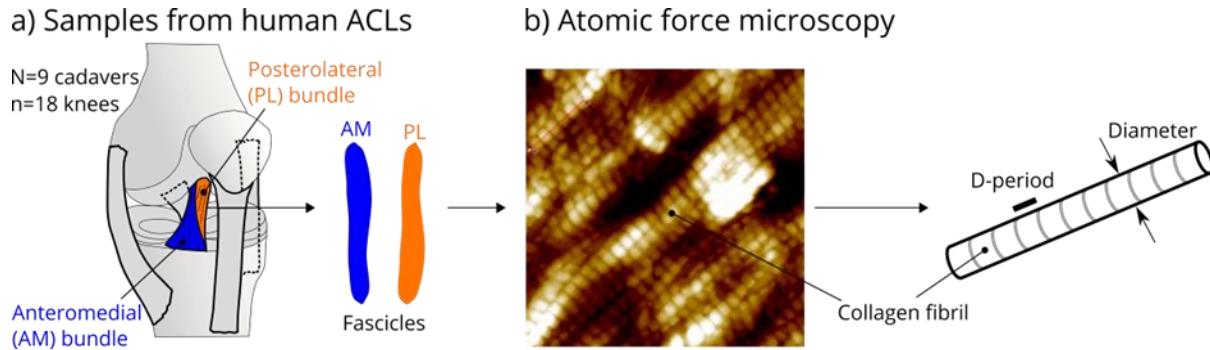


Fig. 1. a) Samples from human anterior cruciate ligaments (ACLs) subjected to b) atomic force microscopy to determine the D-period and diameter of collagen fibrils.

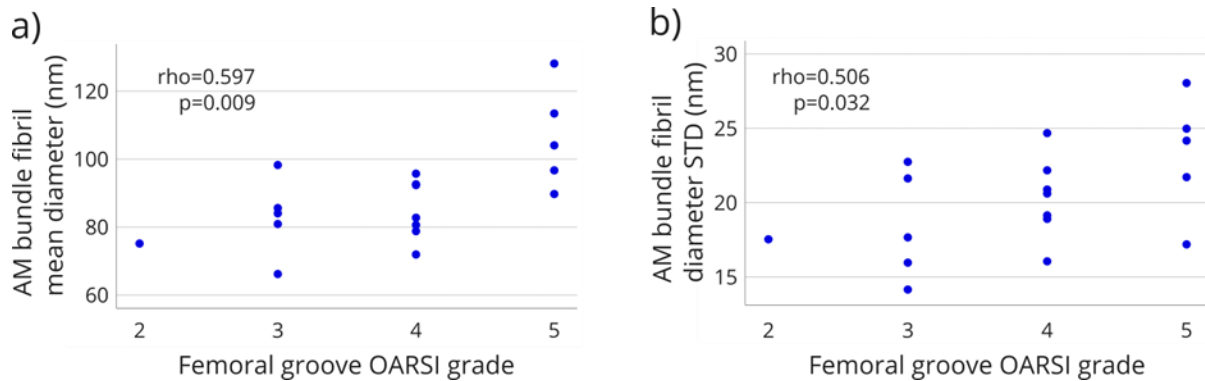


Fig. 2. Correlation between AM bundle a) fibril diameter and femoral groove OARSI grade, and b) fibril diameter standard deviation (STD) and femoral groove OARSI grade.

Po2_7.21

The role of SIRT3 of mitochondrial homeostasis regulation in rotator cuff injury and repair

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Abstract

OBJECTIVES: Rotator cuff injuries have a heavy disease burden, unsatisfactory treatment effects, and unclear internal mechanisms. This study intends to investigate differentially expressed genes that regulate mitochondrial homeostasis to mediate rotator cuff injury repair, verify and screen key signaling pathways, and reveal new potential mechanisms of rotator cuff injury repair and potential therapeutic targets.

METHODS: This study was approved by IACUC. Supraspinatus tendinopathy was induced in both shoulders of mice by inserting a microsurgical clip in the subacromial space for 8 weeks for transcriptomics testing. 21 mitochondrial deacetylase SIRT3 knockout and wild-type mice were induced to supraspinatus tendinopathy. The evaluation indicators included: (1) histological analysis; (2) biomechanical testing; (3) gene expression; (4) mitochondria-related enzyme activity detection; (5) transmission electron microscopy analysis.

RESULTS: The top ten differentially expressed genes were found by transcriptome sequencing analysis of injured and normal tendons. The results of GSEA analysis showed that three mitochondrial-related pathways were enriched in genes highly related to SIRT3. After preliminary verification, it was proposed that the deacetylase SIRT3 regulates Acad9-mediated mitochondrial homeostasis and affects the repair of rotator cuff injury. Histological evaluation: There was no significant difference between the supraspinatus tendon of SIRT3 knockout mice and wild-type mice. The injured tendon after SIRT3 knockout had a significantly increased number of cells, discrete fiber bundles, disordered collagen arrangement, and significantly increased new blood vessels compared with the tendon of wild-type mice. Biomechanical test: There was no significant difference in the supraspinatus tendon of SIRT3 knockout and wild-type mice. The load failure and stiffness of SIRT3 knockout mice were statistically different from those of wild-type mice. Gene expression level: The expression levels of SIRT3 and Acad9 in knockout mice were reduced, and the statistical difference in the injury group was significant. There was no significant difference in the expression level of tendinopathy-related genes between SIRT3 knockout mice and wild-type mice. The expression of tendinopathy-related genes following injury in both SIRT3 knockout mice and wild-type mice changed, confirming that pathological damage occurred in the pathological tendon. After rotator cuff injury. Mitochondrial enzyme-related activity detection and transmission electron microscopy analysis are in progress.

CONCLUSIONS: This study found differentially expressed genes that play a role in rotator cuff tendon mediated by SIRT3-regulated mitochondrial homeostasis and preliminarily verifying that SIRT3 regulates Acad9-mediated mitochondrial homeostasis, affecting rotator cuff injury repair, revealing potential new mechanisms and therapeutic targets for rotator cuff injury repair.

Po2_7.23

Unveiling inflammation dissipation and pro-fibrotic cell populations of subacromial bursa as critical contributors to frozen shoulder by integrating single-cell transcriptomics and spatial transcriptomics

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Abstract

Background: The subacromial bursa is believed to play a significant role in the pathogenesis of FS, particularly in the inflammatory and fibrotic processes. This study aims to explore the cellular microenvironment of the SAB in FS using single-cell RNA sequencing and spatial transcriptomics to identify key pathways and targets for therapeutic intervention.

Methods: We collected SAB samples from FS patients (n=9) through arthroscopic capsular release and from simple rotator cuff injury patients (n=5) through rotator cuff repair from August 2023 to April 2024. Single-cell RNA sequencing, exosome sequencing and spatial transcriptomics were performed on selected samples.

Results: Eight major cell clusters were identified, including skeletal muscle satellite cells, endothelial cells, fibroblasts, mural cells, proliferating cells, T and NK cells, mast cells, and mononuclear phagocytes. In the FS group, there was a significant increase in fibroblasts and a decrease in endothelial and mural cells, indicating a fibrotic and inflammatory environment. Subpopulations of endothelial cells, mural cells, T and NK cells, and fibroblasts were further characterized, revealing distinct phenotypes associated with inflammation and fibrosis. Fibroblasts in the FS group showed upregulation of genes related to ECM synthesis and fibrosis, while endothelial cells exhibited a shift towards anti-inflammatory and fibrotic phenotypes. Macrophage subpopulations were also identified, with some showing pro-inflammatory characteristics and others involved in tissue repair and fibrosis. Spatial transcriptomics revealed the spatial distribution of these cell types, with fibroblasts showing a proliferative and migratory pattern in the FS group.

Conclusions: Our study provides a comprehensive characterization of the cellular microenvironment of the SAB in FS. The findings highlight the complex interplay between fibroblasts and macrophages and their roles in inflammation and fibrosis.

Po2_8.01

Osteochondral tissue regeneration in an orthotopic rat model using gelatin-based cellular implants

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Abstract

Osteochondral defects remain a significant clinical challenge, particularly for young and active patients, as current treatments are often inadequate. Osteochondral tissue engineering offers a regenerative approach by developing biomaterial-based implants that restore native tissue function. In this study, we evaluated gelatin-based hydrogels encapsulating multiple cell types for osteochondral regeneration through *in vitro* and *in vivo* assessments.

Human iPSC-derived chondrocytes (hiPSC-chons), human periosteum-derived cells (hPDCs), and primary human articular chondrocytes (hACs) were embedded in gelatin methacryloyl (GEL-MA INX210, BIO INX) and cultured for up to 21 days. Cell viability, metabolic activity, and gene expression (SOX9, ACAN, COL1A1, COL2A1, COL10A1, RUNX2, OSX, IHH, CNMD) were analyzed. Constructs combining hiPSC-chons (1 or 21 days pre-culture) and hPDCs (21 days pre-culture) were implanted in critically sized osteochondral defects (1.6 mm diameter, depth) in nude rats and evaluated at 4 and 16 weeks post-implantation using histology, immunohistochemistry (collagen type I, II, and human osteocalcin), and contrast-enhanced microcomputed tomography. To quantify the newly formed matrix in the defect site, explants were also analyzed with contrast-enhanced microcomputed tomography.

All cell types exhibited high viability within GEL-MA INX210, with distinct lineage-specific gene expression. hiPSC-chons demonstrated robust chondrogenic marker upregulation (COL2A1, SOX9, ACAN: 25-, 2.5-, and 5-fold, respectively), whereas hPDCs favored hypertrophic and osteogenic markers (COL1A1, COL10A1, RUNX2: 6-, 500-, and 20-fold, respectively). Upon orthotopic implantation, histology showed that the hiPSC-chons produced a cartilage-like matrix high in glycosaminoglycans and collagen type II, especially when constructs were implanted after only 1 day of *in vitro* culture. Bone regeneration was consistent over all conditions and by 16 weeks post-surgery, the volume of mineralized matrix formed was not significantly different from healthy control joints. Also, human osteocalcin was observed, indicating the contribution of the humanized implant to tissue regeneration.

Based on our studies, we suggest that immature hiPSC-chon constructs in combination with *in vitro* matured hPDC constructs are favorable for osteochondral tissue engineering. In a next stage, these constructs will be generated via 3D bioprinting to allow for better adhesion between the both implant types and to form a true mimic of the site of tissue defect.

Po2_8.05

Bridging the Gap in Cartilage Repair: Development of a Human Osteochondral Explant Model for Advanced Regenerative Therapies

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Abstract

Introduction: Joint surface defects are prevalent challenges in orthopedics, trauma care, and sports medicine. The avascularity of articular cartilage limits its natural ability to heal large defects. To enhance treatment strategies for cartilage damage, a deeper understanding of cartilage repair mechanisms is critical. However, developing realistic in vitro models has proven difficult. This study focused on establishing a human osteochondral explant model tailored for investigating cartilage defect treatments using biomaterials or stem cells.

Methods: Osteochondral explants (Ø 10 mm) were harvested from femoral condyles of 17 osteoarthritis patients undergoing knee replacement surgery (N=61 samples). The explants were divided into six groups, each consisting of three samples: no defect, defect only, defect + fibrin glue, defect + ChondroFiller® (meidrix GmbH), defect + fibrin glue + mesenchymal stem cells (MSCs), and defect + ChondroFiller® + MSCs. A biopsy punch (Ø 4 mm) was used to induce defects in the samples. The explants were cultured in ThinCert® inserts (Greiner Bio-One), with separate media for subchondral bone and articular cartilage, for 7, 14, or 21 days. Fresh, defect-free explants (d0) served as controls. After culturing, a series of analyses were performed, including cell viability (MTT assay), DNA content (Hoechst assay), collagen (hydroxyproline assay), and glycosaminoglycan (GAG) levels (DMMB assay), alongside histological and fluorescence microscopy evaluations.

Results: Metabolic activity was sustained in living cells even after 21 days of culture. A 14-day period proved optimal, with DNA content initially decreasing at day 7 but rising up to 2.4-fold in ChondroFiller®-treated groups by day 14, suggesting cell migration. By day 21, DNA levels declined again. Collagen levels mirrored this trend, with ChondroFiller® and MSC-treated explants exhibiting higher collagen content than empty defect controls, indicating MSC differentiation. GAG concentrations were elevated in all treated defect groups by day 14. Notably, DNA and collagen levels showed positive correlations in both ChondroFiller® (p=0.017) and fibrin glue + MSC (p=0.024) groups.

Conclusions: Our results showed that human osteochondral explants are a robust tool for evaluating cartilage repair strategies. The findings provided critical insights into the temporal dynamics between cell-biomaterial interactions, including cell migration, de novo collagen synthesis, and cartilage matrix remodeling, providing a biomimetic platform for future advancements in cartilage defect therapies.

Po2_8.07

Cytoskeleton reorganization rejuvenates senescent chondrocytes via FAK/Dnmt1 pathway

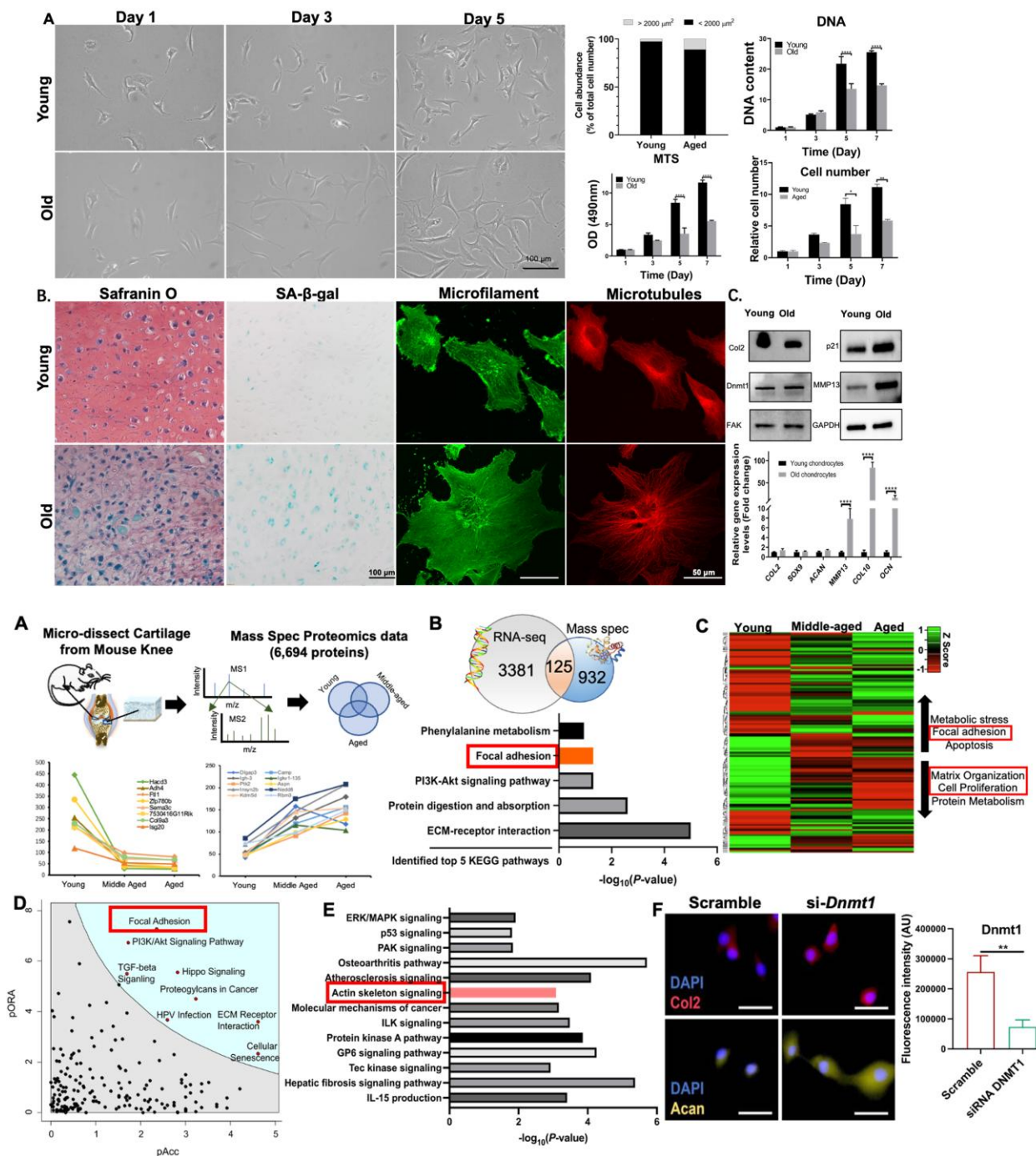
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Abstract

Focal articular cartilage defects are characterized by high morbidity and disability rates. Autologous chondrocyte implantation (ACI) technology, as an effective means to repair cartilage defects, is limited by the senescence of chondrocytes. To enhance the therapeutic effect of ACI, we investigated the mechanism of chondrocyte senescence and identified that reorganizing the cytoskeleton can rejuvenate chondrocytes and enhance their proliferation and cartilage repair capabilities. Through gene sequencing and proteomic analysis, we identified that focal adhesion kinase (FAK) and DNA methyltransferase 1 (Dnmt1) were involved in regulating chondrocyte senescence. Reorganizing the cytoskeleton promoted the nuclear translocation of FAK and reduced Dnmt1 and DNA methylation. Previous studies showed that nuclear FAK controlled the ubiquitination degradation of nuclear proteins. Protein interaction analysis revealed that the E3 ligase Mdm2 interacted with FAK, Dnmt1, and cytoskeletal proteins. Therefore, we hypothesized that cytoskeleton reorganization, by promoting FAK-dependent ubiquitination degradation of Dnmt1, rejuvenated senescent chondrocytes. In this study, we verified the role of cytoskeleton reorganization in rejuvenating chondrocytes and reveal its regulatory FAK/Mdm2/Dnmt1 pathway. We hoped to provide new theories and approaches for promoting cartilage regeneration and optimizing the clinical efficacy of ACI.

AO Orthopaedic Research Summit



Po2_8.08

Detergent-free supercritical CO₂-assisted protocol for the sustainable production of highly preserved decellularized porcine meniscus for orthopedic applications

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Abstract

Meniscus injuries, along with the frequently ensuing condition of osteoarthritis, present a significant burden to the healthcare system [1]. While current treatments such as conservative management aim to alleviate symptoms, surgical approaches—including meniscectomy, meniscus repair, and allograft transplantation—strive for recovery. These interventions often result in suboptimal outcomes, failing to achieve complete and effective meniscal restoration [2]. Meniscus tissue engineering has emerged as a promising alternative, addressing these limitations.

This study aims at developing a decellularized scaffold derived from porcine meniscus—an abundant by-product of daily meat consumption—to restore both the structure and function of the meniscus, thereby promoting long-term recovery. Porcine meniscus undergoes decellularization, a process designed to remove xenogeneic cellular components while preserving the essential extracellular matrix (ECM) [3]. Unlike conventional protocols that rely on harsh detergents, this study employs an innovative detergent-free decellularization approach utilizing a supercritical carbon dioxide (scCO₂)-assisted process. This protocol is environmentally friendly, fast-setting, and cost-effective, offering a significant improvement in sustainability and scalability over traditional methods. The optimized detergent-free protocol demonstrates remarkable efficacy, achieving residual DNA content below 50 ng/mg of dry tissue. Furthermore, it ensures the preservation of ECM matrix architecture and key components including collagen, elastin, and glycosaminoglycans, while maintaining biomechanical performance. *In vitro* cytotoxicity assays revealed no cytotoxicity after direct contact with human fibroblasts.

This innovative detergent-free decellularization process can open new horizons in terms of producing biomimetic meniscus scaffolds that can be further adapted for patient-specific regenerative applications through recellularization using patient-derived cells. Moreover, the decellularized porcine meniscus can be utilized as raw material in various other meniscus tissue engineering approaches, such as 3D printing and hydrogel development, expanding its potential for restoring and regenerating meniscal injuries.

Acknowledgements: The authors would like also to acknowledge the support of Project No. IBEROS+ (0072_IBEROS_MAIS_1_E) - Instituto de Biofabricación en Red para El Envejecimiento Saludable, funded by “Interreg VI A España – Portugal (POCTEP) 2021-2027. JBC is grateful to FCT for the individual Junior Researcher contract (org.doi:10.54499/2022.02781.CEECIND/CP1745/CT0001)

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Po2_8.09

MiRNA modulation for bone-vessel-nerve triad regeneration: A breakthrough in bone defect therapy?

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Abstract

Bone homeostasis is a highly regulated process involving several molecular pathways, but also requiring support from surrounding tissues as vessels and nerves. In bone defects, the bone-vessel-nerve triad is impacted. The regeneration of the bone first requires new innervation followed by angiogenesis and bone formation. While studies mainly investigate how to improve mineralization, a few are focusing on innervation and vascularization as adjuvants to bone regeneration. Recently, new components appeared as regulators of bone homeostasis: microRNAs. For example, miR-214 decreases bone formation by downregulating human mesenchymal stem cells (hMSCs) differentiation and is secreted by osteoclasts to regulate osteoblasts and inhibit bone formation. In this work, we aimed to study the implication of the cluster miR-199a/214 on the bone-nerve-vessel triad.

To assess the effect of miR199a/214 on mineralization, hMSCs and osteoblasts (n=3) were transfected with inhibitor of miR-199a-5p or miR-214, or with a scrambled miRNA (negative control) in calcium-enriched medium (Ca⁺⁺). Mineralization was assessed by Alizarin Red/CPC staining, microRNA expression by qPCR. To assess the effect of miR199a/214 on axon growth, PC-12 and iPSCs were differentiated into neurons (neuronal differentiation medium) and transfected with the same reagents. Axon growth was assessed by immunofluorescence.

We observed that exposure of hMSCs or osteoblasts to Ca⁺⁺ increased mineralization compared to basal medium. Inhibition of miR-214 or miR-199a-5p in Ca⁺⁺-treated osteoblasts or hMSCs, induced higher mineralization compared to scramble. Regarding axon growth, we observed that treatment of PC-12 or iPSCs with neuronal differentiation medium induced axon development. We then showed that the inhibition of miR-199a-5p, particularly, is increasing axon length, axon junction number and axon percentage area compared to scramble-transfected cells in PC12 cells. Moreover, inhibition of miR-199a or miR-214 in iPSCs accelerated their specific differentiation into motor neurons compared to scrambled-transfected cells. *In silico* studies revealed neuron-related mRNAs as potential targets of miR-199a/214.

We highlighted that miR-199a-5p and miR-214 are able to modulate mineralization of hMSCs and osteoblasts. Moreover, these microRNAs also showed the ability to regulate axon growth in transfected PC-12 and iPSCs. The cluster miR-199a/214 is known as modulator of vascular function and could thus contribute to bone remodeling via different ways. For future work, we aim to design a scaffold presenting the inhibitors of miR-199a/214 to implant in a bone defect and improve bone, vessel and nerve regeneration at once for an efficient recovery. With this work we slightly open the door to possible therapeutic approaches for bone diseases.

Po2_8.10

Guiding cell alignment and chondrogenesis for articular cartilage repair

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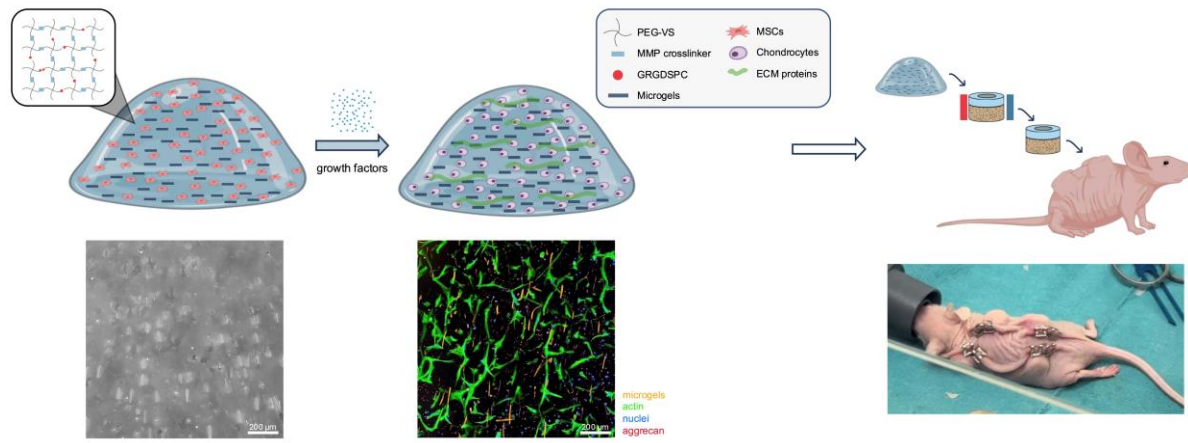
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Abstract

Articular cartilage is a highly specialized tissue with distinct zones differentiated by tissue organization and extracellular matrix (ECM) composition. Each zone plays a critical role in the mechanical and biological functionality of the tissue and is characterized by a specific orientation of both the chondrocytes and the ECM. Current available regenerative strategies are not replicating this zonal architecture and thus do not result in fully functional tissues. The Anisogel is a PEG-based microgel-in-hydrogel system designed to control cell alignment and anisotropic ECM deposition. Magneto-responsive rod-shaped microgels are embedded into a surrounding hydrogel precursor solution and aligned using a weak external magnetic field. Upon crosslinking of the surrounding hydrogel, the position of the microgels is fixed in a specific orientation, thereby guiding anisotropic cell growth and ECM deposition.

In this study, we utilize the Anisogel system to evaluate the effect of the presence of aligned microgel rods for the differentiation of human bone marrow-derived mesenchymal stromal cells (MSCs) into chondrocytes. We first optimized the hydrogel for chondrogenesis by varying the polymer concentration in the hydrogel and the concentration of Arginine-Glycine-Aspartic acid (RGD) peptide sequence. We observed increased cartilaginous marker deposition for lower hydrogel stiffness and increased RGD content. When incorporating the magneto-responsive microgels into the optimized hydrogel, we found that the highest cell alignment was achieved with larger microgels (10x10x100 μm^3). Interestingly, we found that the deposition of articular cartilage-specific markers, such as collagen II and aggrecan, was significantly increased in samples with aligned microgels compared to those with unaligned ones. This indicates that the spatial orientation of microgels plays a key role in promoting a cartilage-like ECM deposition. Furthermore, initial *in vivo* studies were conducted using this system in a semi-orthotopic mouse model. In this model, artificial defects are created in bovine osteochondral plugs and filled with the Anisogel before implantation. The results are expected soon and will be

included in the final poster. The results obtained so far highlight the potential of Anisogel in supporting zonal cartilage regeneration through controlled anisotropic cell and ECM alignment.



Development of a microgel-in-hydrogel system (Anisogel) for zonal cartilage regeneration, consisting of a PEG-based hydrogel with aligned rod-shaped microgels and bone marrow-derived mesenchymal stromal cells (MSCs). Growth factors induce chondrogenic differentiation, while microgel alignment guides cell orientation and anisotropic extracellular matrix (ECM) deposition. For in vivo evaluation, Anisogel is incorporated into osteochondral plugs with artificial defects and implanted subcutaneously in semi-orthotopic mouse model.

Po2_8.11

Adaptation of Near Infrared Spectroscopy for In Situ Monitoring and Optimisation of Tissue Engineered Cartilage Growth.

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Abstract

Articular cartilage lesions may remain clinically silent and unnoticed for decades while morphologically progressing to osteoarthritis. As current cartilage repair procedures often fail to restore long-term physiological tissue function, tissue engineering (TE) presents an alternative for creating viable replacement tissues. This project aims to develop novel protocols for monitoring cartilage TE using near infrared spectroscopy (NIRS), an optical spectroscopic technique that has gained interest for evaluating connective tissue integrity (Afara et al., 2021).

We conducted cartilage TE using human bone marrow mesenchymal stem/stromal cells seeded in gellan gum and gelatin methacrylate (GelMA) hydrogels. Constructs were cultured for up to 4 weeks in established serum-free chondrogenic differentiation medium. NIRS spectra were acquired every 24h, directly on the TE constructs using a custom setup that does not interfere with the TE process. Constructs were harvested at various time points (1, 3, 7, 14 and 28 days) and subjected to biomechanical testing and extensive tissue reference analysis to determine key biochemical constituents, such as glycosaminoglycans, alkaline phosphatase and water content, which are important biomarkers of tissue growth and development. Lactate dehydrogenase (LDH) activity in the conditioned medium was measured as an indication of cell damage. The relationship between the spectra and culture duration was assessed using machine learning models.

Results of spectral data analysis show that NIRS can accurately estimate the extracellular matrix (ECM) composition. We observed that the cells seeded in gellan gum hydrogel produce more cartilage-specific ECM compared to cells seeded in GelMA. A higher cell damage in GelMA compared to gellan gum was evident, as indicated by the high release of the LDH enzyme. This may be due to exposure to ultraviolet light during crosslinking of GelMA, which may have affected its cell differentiation and altered production of ECM. Our preliminary analyses suggest that NIRS is capable of accurately estimating TE cartilage properties in real-time. We aim to adopt this optical monitoring approach for assessing the effect of mechanical loading on tissue growth in a bioreactor.

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Po2_8.13

INJECTABLE POLYMERIC HYDROGEL FOR ADVANCED CELL DELIVERY IN CARTILAGE REGENERATION

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Abstract

Injectable hydrogels are increasingly recognized as versatile tools in tissue engineering and regenerative medicine due to their minimally invasive application, adaptable properties, and ability to support cell viability [1-3]. A key requirement for their clinical use is a well-controlled, reversible sol-gel transition under physiological conditions, enabling precise cell delivery while maintaining functionality. This study focuses on the development of dynamic polysaccharide-based hydrogels that combine ampholyte chitosan and clickable dextran derivatives with gelatin to enhance their mechanical stability and biocompatibility.

The hydrogels exhibit dynamic shear-thinning behavior and self-healing properties, ensuring injectability through various needle sizes while preserving their structural integrity. Viability tests using 3T3 fibroblasts confirm their non-cytotoxic nature, highlighting their potential as scaffolds for cell delivery. Detailed physicochemical characterization allowed the identification of optimal formulations for in vivo testing. These findings underscore the potential of these hydrogels as advanced platforms for cell delivery in tissue engineering and regenerative medicine applications.

Acknowledgment. This work is the result of the project implementation Advanced bioactive hydrogel scaffolds for regenerative medicine (ABSACARM), ITMS2014+: 313011BWL6 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund. This work was partially supported by APVV-22-0565 and APVV-22-0568.

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Po2_8.14

A New In Vitro Model of Inflammation and Endothelial Dysfunction to Investigate the Effects of Ion-Releasing Fabrics on Musculoskeletal Inflammatory Processes

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Abstract

Ion-releasing fabrics have shown promising results in managing a range of musculoskeletal conditions by leveraging the controlled release of bioactive ions to reduce inflammation and promote tissue repair, offering a non-invasive therapeutic alternative. Despite their innovative potential, the underlying cellular mechanisms through which these fabrics exert their effects remain poorly understood. Clinical evidence suggests that fabrics infused with negative ions and semiconductor materials like germanium can modulate inflammation, enhance blood flow, and mitigate oxidative stress by utilizing the therapeutic properties of charged particles, far-infrared radiation, and electromagnetic waves.

To bridge the gap between clinical observations and mechanistic insights, this study introduces an advanced in vitro model of inflammation and endothelial dysfunction (named InRED) developed in both 2D and 3D culture formats. The model was designed to assess the impact of ion-releasing fabrics on cell viability, as well as the production of pro-inflammatory and anti-inflammatory cytokines. Co-cultures of pre-stressed primary human endothelial cells (HUVECs) and THP-1 monocytes were treated with the fabrics to investigate complex cytokine expression profiles and cellular behaviors. Additionally, human tendon cells (hTCs) derived from both healthy and pathological tendon explants were incorporated into the system to explore the fabrics' effects on inflammation, senescence, and fibrosis.

Po2_8.17

Macromolecular crowding in the development of advanced therapy medicinal products for orthopaedic indications

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Abstract

In vivo, cells reside in a crowded, by a diverse range of macromolecular species, extracellular milieu. In contrast to nature's paradigm, *in vitro* eukaryotic cell cultures are conducted in deprived of macromolecules culture media. In such non-crowded environments biological processes occur very slowly, if they are initiated at all. As a result, the development of advanced therapy medicinal products requires prolonged culture times (4-24 months) that are associated with very high manufacturing costs and loss of cellular phenotype. Macromolecular crowding (MMC) is a biophysical phenomenon based on the excluded volume effect theory, according to which two molecules cannot occupy the same space at the same time. In eukaryotic cell culture, the utilisation of MMC induces volume exclusion, which reduces diffusion and promotes molecular interactions. As a result, under MMC conditions, advanced therapy medicinal products can be developed rapidly (1-2 weeks). This talk will advocate the utilisation of MMC in both permanently differentiated and mesenchymal stromal cell cultures for the development of tissue engineered medicines for orthopaedic applications (e.g. tendon, cartilage).

Acknowledgements. This worked has emanated from research supported by Research Ireland (grant number 19/FFP/6982). This work has also received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant number 866126).

Po2_8.18

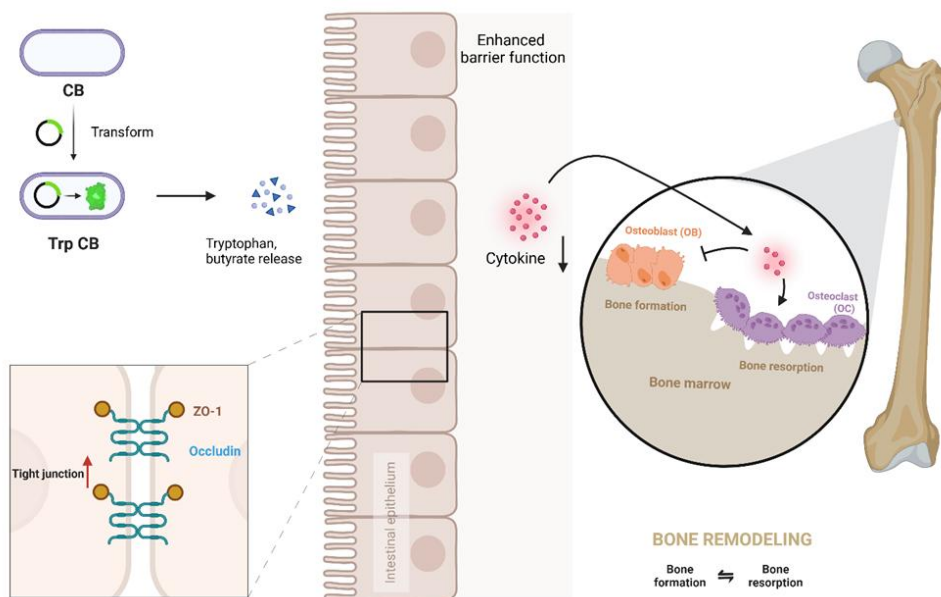
The Study of Osteoporosis Induced by Chronic Colitis Through the Tryptophan Metabolism Pathway

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Abstract

The relationship between gut microbiota and host health and disease is intricate, with microbiota-derived metabolites playing a crucial role in the gut-organ axis. In this study, we observe evident osteoporosis in mice models with intestinal inflammation. Significantly decreased levels of microbial metabolites, particularly tryptophan derivatives are noted, which induced intestinal epithelial barrier dysfunction. Under pathological conditions, compromised intestinal barrier integrity leads to bone inflammatory responses, culminating in pathological osteoporosis. Through supplementation of tryptophan by genetically engineered probiotics, we effectively repair damaged intestinal barriers in colitis mice and mitigate bone loss, indicating the link between chronic colitis and osteoporosis. Furthermore, supplementation of tryptophan with engineered probiotics in an aged mouse model alleviates age-related osteoporosis. These findings provide a potential therapeutic strategy for osteoporosis, a chronic disease with increasing prevalence in aging societies.



Po2_8.19

The Role of GDF5 in Enhancing Cell Survival on Silk Scaffolds for Intervertebral Disc Regeneration

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Abstract

Introduction. Low back pain (LBP) is a major global burden of disability, often caused by degeneration of the intervertebral disc (IVD). Current treatments focus on symptom management and fail to offer long-term regenerative solutions. Silk fibroin has emerged as a promising biomaterial for orthopedics in general but also for IVD repair due to its excellent biocompatibility and tunable mechanical properties. This study investigated the potential of fiber-based additive manufactured (FAM) silk scaffolds vs a fleece silk fiber composite we investigated previously (Frauchiger et. al) with and without growth and differentiation factor 5 (GDF5) added to the medium.

Methods. Human bone-marrow-derived mesenchymal stromal cells (hMSCs) collected from vertebrae during spinal surgery were expanded up to P2-P3 in α modified Eagle Medium (MEM). 600k cells were seeded on each silk scaffold (5×5 mm for fleece; 5 mm diameter × 3 mm height for FAM) and cultured in low-glucose Dulbecco's Modified Eagle Medium (LGDMEM) and allowed to settle for 24h; the medium was exchanged for a chondrogenic inductive medium, i.e., High glucose Dulbecco's Modified Eagle Medium (HGDMEM)+ 1% ITS+ (Insulin, transferrin, selenium, bovine serum albumin, and linoleic acid +1% non-essential amino acids, 172 μ M L-ascorbic-acid 2 phosphate, 100 nM dexamethasone, 2% FCS and 100 ng/mL GDF5. All samples were cultured at standard culture conditions. Cell viability/ cytocompatibility was assessed on days 7 and 14 using Live/Dead staining. Confocal laser scanning microscopy (cLSM) was performed using z-stacks and tile scans of the scaffolds.

Results. Live/Dead staining showed >90% of viable cells on both scaffolds at day 7 but <20% of cells viable at day 14. GDF5 significantly enhances cell viability and attachment on both engineered silk and FAM silk scaffolds on day 7. Engineered silk with GDF5 supports better cell survival over time whereas FAM silk alone is less supportive at day 14. However, its performance improves with GDF5, highlighting the importance of bioactive agents for

scaffold-based tissue engineering. Overall there is a reduction in the number of viable cells on day 14 as compared to day 7.

Conclusion. The study suggests while GDF5 enhances early cell viability, it fails to maintain long-term survival, indicating that scaffold properties also need to be considered for the sustained growth of cells.

Acknowledgments This Research was financed by a Weave Agency Grant of the Swiss National Science Foundation (# 320030E_224175) and the German Research Society (Deutsche Forschungsgemeinschaft, DFG, # 437213841). We thank Yamina Baumgartner for assistance with assays.

Live/ Dead staining on day 7 and day 14

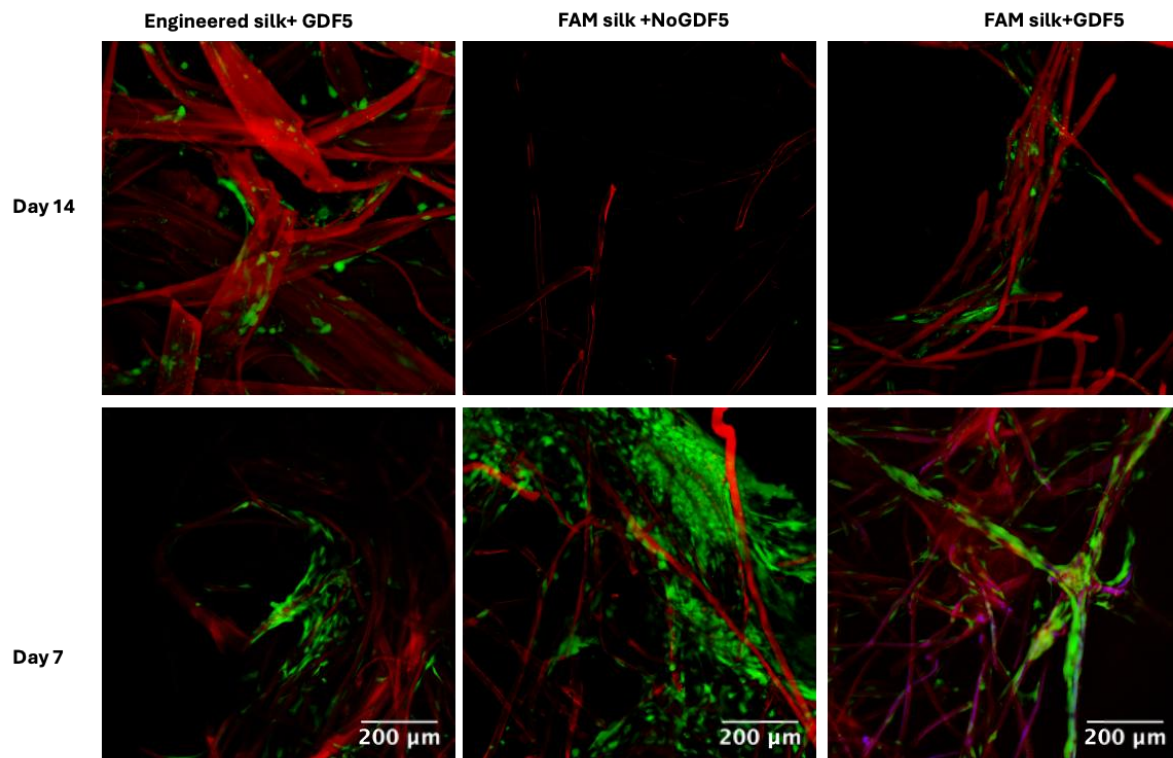


Figure: Live/Dead staining (Z-stack fluorescence images) of engineered silk and FAM (Fiber Additive Manufacturing) silk scaffolds cultured with or without Growth Differentiation Factor 5 (GDF5, 100 ng/mL) on Day 7 and Day 14 (n=1). The images show >90% cell viability on both scaffolds at day 7, dropping to <20% by day 14. GDF5 increases early cell viability and attachment on engineered and FAM silk. Additionally, Engineered silk+GDF5 maintained better long-term survival than FAM silk alone, though GDF5 improved FAM performance also. Bioactive factors like GDF5 are crucial, but overall viability declined by day 14. The Green fluorescence (Calcein-AM) indicates viable cells, while red fluorescence represents dead cells and/or scaffold autofluorescence. Scale bar = 200 μm.

Po2_9.01

Tracking Recovery: From Injury to Recovery with Motion Capture and Sensor Technology

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Abstract

Background: Advanced sensor insoles and motion capture technology can significantly enhance the monitoring of patient's rehabilitation progress with distal tibial fractures [1], [2]. This study utilizes the potential of these innovative tools to provide a more comprehensive assessment of a patient's gait and weight-bearing capacity following surgical intervention, offering a possibility for improved patient outcomes.

Methods: A patient who underwent distal medial tibial LCP surgery in August 2023 and a subsequent revision surgery involving the insertion of an intramedullary nail in December 2023 was meticulously monitored over 12 weeks. Initial assessments in November 2023 revealed pain upon full weight-bearing without crutches. Post-revision, precise weekly measurements were taken starting two days after surgery, instilling confidence in the ability to accurately track the patient's progress from initial crutch-assisted walking to full recovery.

Objectives: The study aimed to evaluate the hypothesis that the approximation and formation of a healthy gait curve are decisive for healing. Specifically, it investigated whether cadence, imbalance factors, and ground reaction forces could be significant indicators of the healing status and potential disorders.

Results: The gait parameters, cadence, factor of imbalance, and ground reaction forces significantly correlate with the patient's recovery trajectory. These metrics allow for the early identification of deviations from expected healing patterns and facilitate timely interventions, underscoring the transformative potential of these technologies in patient care.

Conclusion: Integrating sensor insoles and motion capture technology offers a promising approach for monitoring the recovery process in patients with distal tibial fractures. This method provides valuable insights into the patient's healing status, potentially predicting and addressing healing disorders more effectively. Future studies are recommended to validate these findings across a larger cohort and explore the possibility of integrating these technologies into clinical practice.

REFERENCES. [1] Orth, M., et al., *Simulation-based prediction of bone healing and treatment recommendations for lower leg fractures: Effects of motion, weight-bearing and fibular mechanics*, *Front. Bioeng. Biotechnol.*, 11(February), 1–13. (2023); [2] Warmerdam, E. et al., *Gait Analysis to Monitor Fracture Healing of the Lower Leg*, *Bioeng.* (Vol. 10, Issue 2). MDPI. (2023)

Po2_9.02

Digital monitoring of patients undergoing orthopedic deformity correction: feasibility and patient acceptability

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Abstract

Introduction. Integrating real-time monitoring technology and asynchronous communication pathways to track patient health after orthopedic surgery may enhance patient care and provide timely data for necessary interventions. However, many healthcare technologies face challenges when implemented in clinical practice. Therefore, interventions need to be tested for feasibility and acceptability.

Aim. This study assesses the feasibility and patient acceptability of a monitoring system including an activity tracker, a pain and medication tracking app, and a communication tool.

Methods. The monitoring system comprises 1) Activity tracker (SENS Motion, SENS Innovation Aps), 2) App for registering pain and medication intake (OrtoApp, Alexandra Institute), and 3) a simple messenger-like communication tool for asynchronous communication between patient and their healthcare team (LetDialog, VISMA). Retention strategies include continuously monitoring participants, providing activity feedback, and sending reminders for data upload. Eligible participants are patients undergoing lower limb deformity correction at Aalborg University Hospital (October 2023–December 2024). Patients are included four weeks prior to surgery, and monitoring continues for three months after surgery. Feasibility is assessed by recruitment success, compliance with sensor wearing time, adherence to upload of data in OrtoApp, and usage data of LetDialog. Acceptability is explored by semi-structured individual interviews with participants, based on a theoretical framework of acceptability.

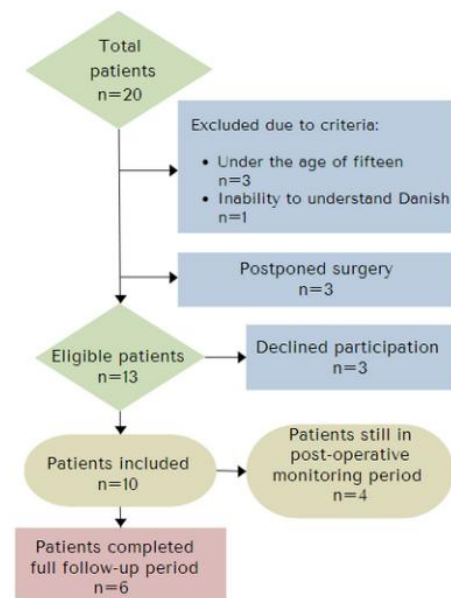


Figure 1 - Flowchart illustrating the inclusion and progression of participants through the study

Results. Six patients (female n= 3, male n=3) have concluded the three-month postoperative monitoring and follow-up period (Figure 1). Four of six patients used the sensor consistently across all days (100%) during both the preoperative and postoperative

periods. One patient used the sensor 100% of the preoperative period and 92% of the postoperative period. One patient used the sensor for 98% of the preoperative period and 100% for the postoperative period (Figure 2). The median (range) proportion of days patients reported in the OrtoApp was for pain 47% (30% - 92%) and for medication intake 10% (0% - 49%). The median (range) of treatment-specific contacts from patients in LetDialog was 5 (2 - 21). Participants expressed perceptions of effectiveness, especially with the use of LetDialog. However, they also reflected upon burdens specifically related to technical challenges and a lack of notifications and feedback systems in SENS and OrtoApp. Overall, patients reported feeling safe and close to their healthcare team through the use of the monitoring system.

Conclusion. Digital monitoring of patients undergoing deformity correction seems both feasible and acceptable. Larger scale studies are needed to examine whether real-time patient monitoring also can improve patient outcome.

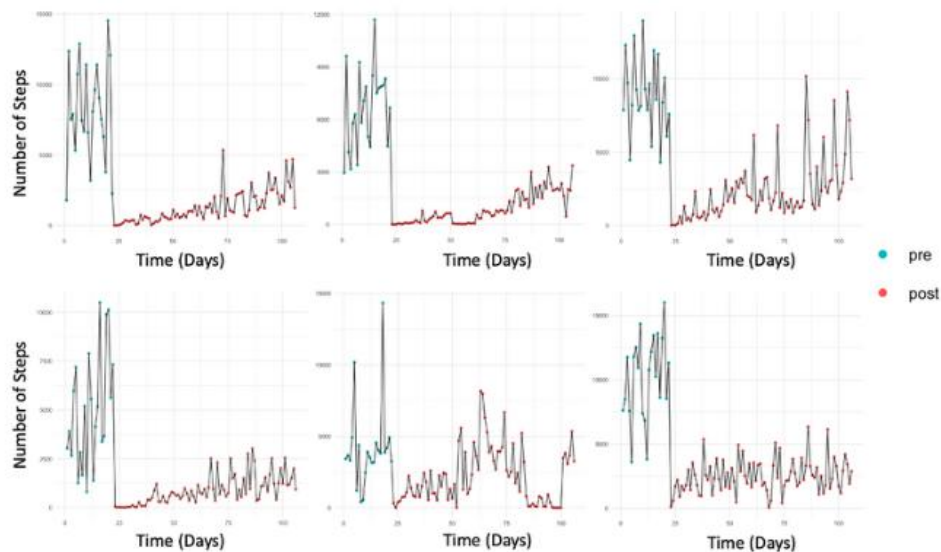


Figure 2 - For each of the six patients is shown the relationship between patient activity in steps (pre- and post surgery) and sensor wearing time (days)

Po3_1.04

Vertebral fracture prediction from MRI-based vertebral bone quality scores in postmenopausal women: A longitudinal cohort study

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Abstract

Objective: The study aims to assess the lumbar MRI-based vertebral bone quality (VBQ) score and dual energy x-ray absorptiometry (DXA) T score in a longitudinal cohort of postmenopausal women and to evaluate the performance of these scores in predicting VF over 5 years of follow-up.

Summary of Background Data: VBQ scores can be used for osteoporosis screening and identification of vertebral fractures (VFs). However, due to limitations in prior cross-sectional studies, definitive conclusions about the causal associations between the VBQ score and incident VF have not been drawn.

Methods: Postmenopausal women with baseline DXA and lumbar MRI data were enrolled. Follow-up assessments were conducted over 5 years after the baseline examination; both anteroposterior- and lateral-view thoracolumbar spine radiographs were captured to evaluate incident VF. At baseline, correlation analyses between the VBQ score and osteoporosis or preexisting VF were performed. A Cox regression model for multivariate logistic regression was used to analyze the risk factors for incident VF. The hazard ratio (HR) was calculated.

Results: 137 postmenopausal women were included. The VBQ score was moderately correlated with lumbar bone mineral density at baseline ($r=0.4$, $p<0.001$). Patients with incident VF had significantly lower T scores (-3.4 vs. -1.87 , $p<0.001$) and higher VBQ scores (4.62 vs. 4.03 , $p<0.001$) at baseline than those without incident VF. After adjusting for age, weight, creatinine clearance, and alkaline phosphatase levels, the HRs for the VBQ score and T score were 1.93 and 0.71, respectively. After adjusting for the T score, an increased VBQ score was independently associated with an increased risk of incident VF, with an HR of 1.87.

Po3_1.10

ECM degradation-driven accumulation of cytotoxic Vδ1 T-cells in Modic type 1 changes

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Abstract

INTRODUCTION: Modic type 1 changes (MC1) are associated with chronic low back pain (cLBP). Despite many studies pointing to the role of inflammation in MC1, a comprehensive investigation of the immune system in MC1 lesions is missing.

OBJECTIVE: To investigate the immunological background of MC1 in relation to degeneration of the adjacent intervertebral disc.

METHODS: Vertebral bone marrow aspirates were collected from cLBP patients undergoing lumbar spinal fusion, and mononuclear cells were isolated. Cells (n=22, MC1+intra-patient control=8+8, control patients=6) were immunophenotyped by massively parallel flow cytometry. T-cell functional properties were analyzed by flow cytometry: 1.) ex vivo analysis of Granzyme B, 2.) Cytokine release after *in vitro* stimulation with Brefeldin and PMA/ionomycin, 3.) Proliferation in response to IL-15 followed by decreased CFSE fluorescence. Single-cell RNA-sequencing: MC1 and control biopsies (n=4+4) were enzymatically digested, neutrophils depleted, and ~10'000 cells sequenced. Extracellular matrix protein analysis: Intervertebral discs were analyzed by LC-MS/MS. Cartilage oligomeric matrix protein (COMP) was quantified in bone marrow plasma with ELISA. COMP functional assay: bone marrow-derived mononuclear cells were treated with COMP, and cytokine production was analyzed using flow cytometry.

RESULTS: Immunophenotyping revealed accumulation of Vδ1 T-cells in MC1 (Fig.1A,B) and dysregulated expression of surface markers on Vδ1 T-cells in MC1 (Fig.1C). Most Vδ1 T-cells in MC1 expressed NKp80 (72% vs. 40% in inpatient control) and NKp80 was more expressed on Vδ1 T-cells than on other T-cells (Fig.1D). NKp80⁺ Vδ1 cells expressed more Granzyme B and, upon stimulation, produced more IFNγ and TNF than other T-cells (Fig.1E). Vδ1 T-cells also expressed more IL15Rα than other T-cells (Fig. 1F), and IL-15 selectively induced their proliferation (Fig.1G). IL-15 levels in bone marrow plasma correlated with Vδ1 frequency (R=0.84). Single-cell RNA-sequencing revealed that IL15 was expressed predominantly in CD16⁺ monocytes, and that CD16⁺ monocytes accumulated in MC1 (Fig.1H). COMP-activated CD16⁺ monocytes in-vitro. COMP was enriched in discs adjacent to MC1 and in MC1 bone marrow (Fig.1I,J,K).

CONCLUSION: We discovered an accumulation of cytotoxic and pro-inflammatory V δ 1 T-cells in MC1 lesions that might be linked to degeneration of the adjacent intervertebral disc. Their proliferation was closely related to marrow levels of IL-15, and they represented the only T-cell subset that proliferated in response to IL-15. A subset of CD16⁺ monocytes accumulating in MC1 was discovered as the primary source of IL-15 in MC1, and we revealed that they can be activated by disc matrix-derived protein COMP, which also accumulates in MC1.

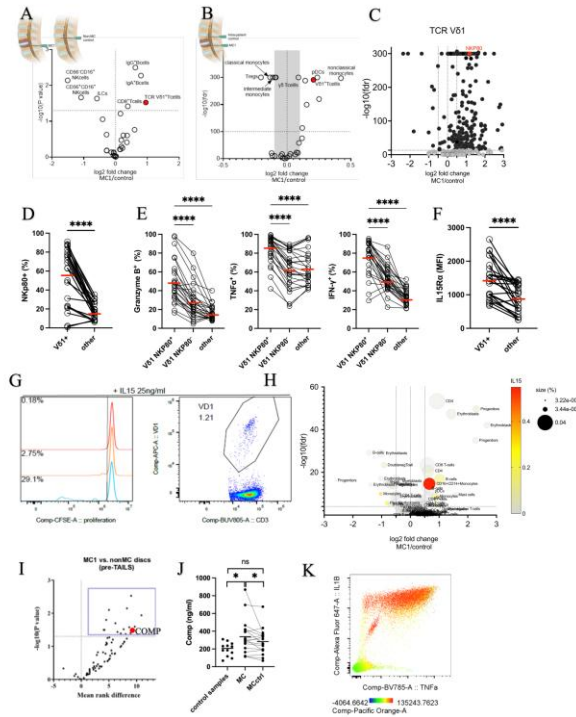


Figure 1. A.) Comparison of immune cell subset presence in MC1 patients and patients without MC1 based on flow cytometry screening of bone marrow aspirates. B.) Comparison of immune cell subset frequencies in MC1 lesions and intrapatient controls based on flow cytometry screening of bone marrow aspirates. C.) Changes in the expression of surface markers on T-cells from MC1 and control tissue. Markers mentioned in the text are highlighted in red. D.) Comparison of NKp80 expression between T cell subsets. E.) Comparison of Granzyme B, IFN γ , and TNF α expression between NKp80⁺ V δ 1 T-cells and other T-cells. F.) Comparison of CD215 expression between T cell subsets. MFI=Median Fluorescence Intensity G.) The proliferation of CD4⁺ $\alpha\beta$ T-cells, CD8⁺ $\alpha\beta$ T-cells, and V δ 1 T-cells in response to IL15. H.) Comparison of clusters of immune cell presence in MC1 lesions and intrapatient control based on single-cell RNA seq of bone marrow biopsies. Expression of IL15 RNA is shown as color code. I.) Comparison of protein presence in intervertebral discs of MC1 and control patients using mass spectrometry. J.) Comparison of COMP levels in bone marrow plasma in MC1 lesions, intrapatient controls, and control patients by ELISA. K.) Activation of PBMCs by 20ug/ml of COMP. Expression of TNF α , IL1 β , and IL14 was followed by flow cytometry. The expression of CD14 is depicted as a color code.

Po3_1.01

Exploring the 3D architecture of native and stained human intervertebral discs through micro-CT

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Abstract

Background. The disruption of normal intervertebral disk (IVD) structure and its degeneration significantly contributes to the onset of low back pain [1]. To date, the human IVD 3D microstructure characterization has been confined to imaging the annulus fibrosus (AF) [2] and the subchondral endplate [3]. Our study aimed to propose a step-wise approach for the complete morphological investigation of human IVDs at various tissue processing stages using a non-destructive micro-CT analysis. This approach allowed detailed 3D visualization of IVD's anatomical components. Additionally, the method provided a precise quantification of lesions and calcifications within the disk, which affect the integrity of its microstructure and thus its functional and biomechanical characteristics [4].

Methods. The IVDs (n = 14) were examined using the micro-CT EasyTom XL CT scanner (RX Solutions, Chavanod, France) with a voxel size of 40 µm. Each sample was scanned label-free (both frozen and thawed) and after formalin fixation and staining of the tissue in an iodine-based contrast agent solution. The calcifications and lesions within the tissue were identified with an automatic segmentation approach using the software Avizo (Thermo Fisher Scientific, MA, USA). The sample volumes were computed and compared for each processing stage. In addition, the degree of contrast enhancement has been defined by calculating the Hounsfield Units (HU) relative to the acquired CT values.

Results. The analysis of frozen samples provided valuable insight into the alternate laminal pattern of the AF. Thawed samples allowed a quantitative analysis of lesions and calcifications in the native IVD. Formalin fixation distorted the morphology of the tissue due to the extrusion of the nucleus pulposus (NP). Iodine staining provided a homogeneous increase in contrast and simultaneous visualization of the IVD anatomical components.

Conclusion. Examining frozen, thawed, and chemically fixed-stained IVD tissues using micro-CT revealed distinctive microstructural information, presenting different benefits for each stage. The systematic methodology used in this study aims to define the trade-off between contrast enhancement and processing artifacts. This approach represents a valuable option for a 3D morphological investigation of the sample, especially when combined with biomechanical testing due to its non-destructiveness.

[1] Diwan A D, (2022) *JOR Spine* [2] Stein D, (2021) *Sci Rep*; [3] Rutges J P, (2011) *Osteoarthritis Cartilage* [4] Whatley B R, (2012) *Materials Science and Engineering*

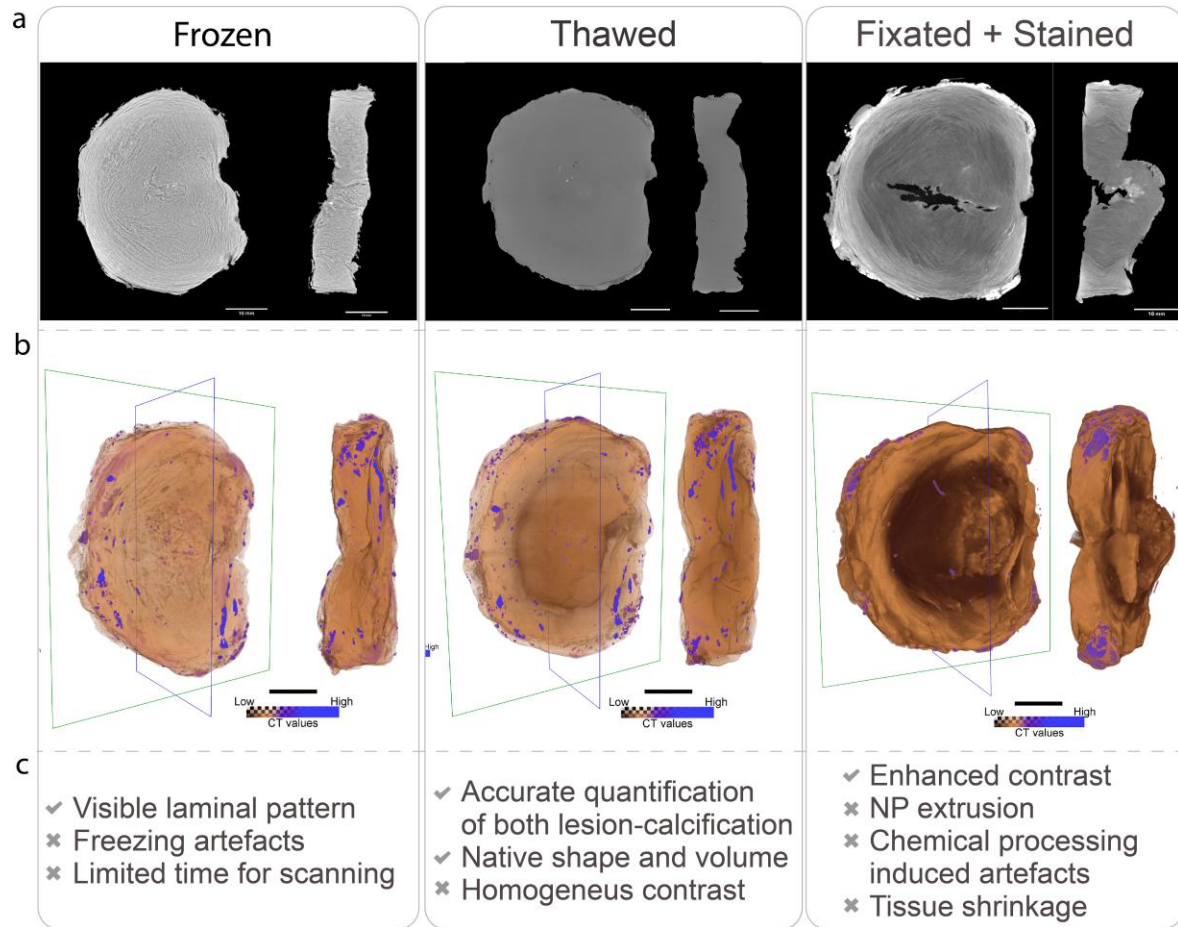


Fig. 1. Axial and coronal micro-CT 2D sections (a) and corresponding 3D models (b) of the IVDs. (c) Benefits and drawbacks. Scalebar 10 mm.

Po3_1.03

A Degradable Nanofibrous Scaffold Of Poly(E-Caprolactone-Co-Lactide)For Annulus Fibrosus Regeneration

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Abstract

The intervertebral disc herniation is a significant cause of low back pain, counting up to 32% of the cases. Previously, we showed the potential of an implant made of poly(ϵ -caprolactone) (PCL) for AF defect closure. However, the slow in vivo degradation of PCL might hinder AF regeneration. Here, we hypothesized that accelerating PCL degradation kinetics by incorporating a lactide polymer known for its rapid degradation enhances AF regeneration.

A Poly(ϵ -caprolactone-co-lactide) (PCLA), was synthesized with various molar percentages of lactide (10, 20, and 30 %) and structures (copolymer or blend). These polymers were electrospun into sheets of aligned nanofibers. Then a multi-lamellar 3D implant was assembled by stacking the PCLA sheets and incorporating an anchoring system (wings that are glued on the adjacent vertebrae). The thermal, mechanical, and structural properties of the novel polymers were analyzed, including the impact of gamma-irradiation sterilization and their in vitro degradation over 6 months. Next, the cytocompatibility of the implant was assessed in vitro with ovine AF cells and ex vivo using a bovine tail disc model. Finally, the implant's regenerative potential was assessed in a sheep model of simulated herniation (n=4).

PCLA sheets had a Young's Modulus ranging from 20 to 37 MPa, close to that of AF tissue. Moreover, gamma irradiation (25 kGy) caused a Young's Modulus increase by 21-31 MPa when lactide > 20%. The 6-months in vitro degradation study of PCLA showed a molar mass loss of $70 \pm 0.4\%$ for the copolymer and $53 \pm 17\%$ for blends. Furthermore, scanning electron microscopy revealed fiber breakage in the copolymer. Furthermore, PCLA sheets guided the in vitro alignment and proliferation of ovine AF cells and maintained the expression of AF markers for up to 2 weeks (collagen type I and type II). Ex vivo, the multi-lamellar implant

was maintained within a full-thickness annular defect (4mm biopsy punch) of bovine tail discs during 4 weeks of culture, and initial cell infiltration into the implant was observed. In vivo, despite instances of implant dislocation, disc hydration was preserved at one-month. Interestingly, substantial cellular infiltration and AF-like tissue ingrowth were evidenced within the implant layers.

This study confirmed the in vitro faster degradation kinetics of PCLA and the affinity of AF cells for the nanofibrous implant. It also highlights the ability of the newly designed multi-lamellar implant to instruct AF tissue regeneration. Future work will evaluate the long-term efficacy (6 months) in our sheep model.

Po3_1.04

Vertebral fracture prediction from MRI-based vertebral bone quality scores in postmenopausal women: A longitudinal cohort study

Tao Chen, Zhiyun Wang, Wentao Lin, Xiaoqian Wang, Houjie Yin

Shunde Hospital, Southern Medical University (The First People's Hospital of Shunde Foshan), Foshan, China

Abstract

Objective: The study aims to assess the lumbar MRI-based vertebral bone quality (VBQ) score and dual energy x-ray absorptiometry (DXA) T score in a longitudinal cohort of postmenopausal women and to evaluate the performance of these scores in predicting VF over 5 years of follow-up.

Summary of Background Data: VBQ scores can be used for osteoporosis screening and identification of vertebral fractures (VFs). However, due to limitations in prior cross-sectional studies, definitive conclusions about the causal associations between the VBQ score and incident VF have not been drawn.

Methods: Postmenopausal women with baseline DXA and lumbar MRI data were enrolled. Follow-up assessments were conducted over 5 years after the baseline examination; both anteroposterior- and lateral-view thoracolumbar spine radiographs were captured to evaluate incident VF. At baseline, correlation analyses between the VBQ score and osteoporosis or preexisting VF were performed. A Cox regression model for multivariate logistic regression was used to analyze the risk factors for incident VF. The hazard ratio (HR) was calculated.

Results: 137 postmenopausal women were included. The VBQ score was moderately correlated with lumbar bone mineral density at baseline ($r=0.4$, $p<0.001$). Patients with incident VF had significantly lower T scores (-3.4 vs. -1.87 , $p<0.001$) and higher VBQ scores (4.62 vs. 4.03 , $p<0.001$) at baseline than those without incident VF. After adjusting for age, weight, creatinine clearance, and alkaline phosphatase levels, the HRs for the VBQ score and T score were 1.93 and 0.71, respectively. After adjusting for the T score, an increased VBQ score was independently associated with an increased risk of incident VF, with an HR of 1.87.

Po3_1.07

Noggin Expression in Human Nucleus Pulposus Cells: A Comparative Study of Degenerative and Healthy Intervertebral Discs

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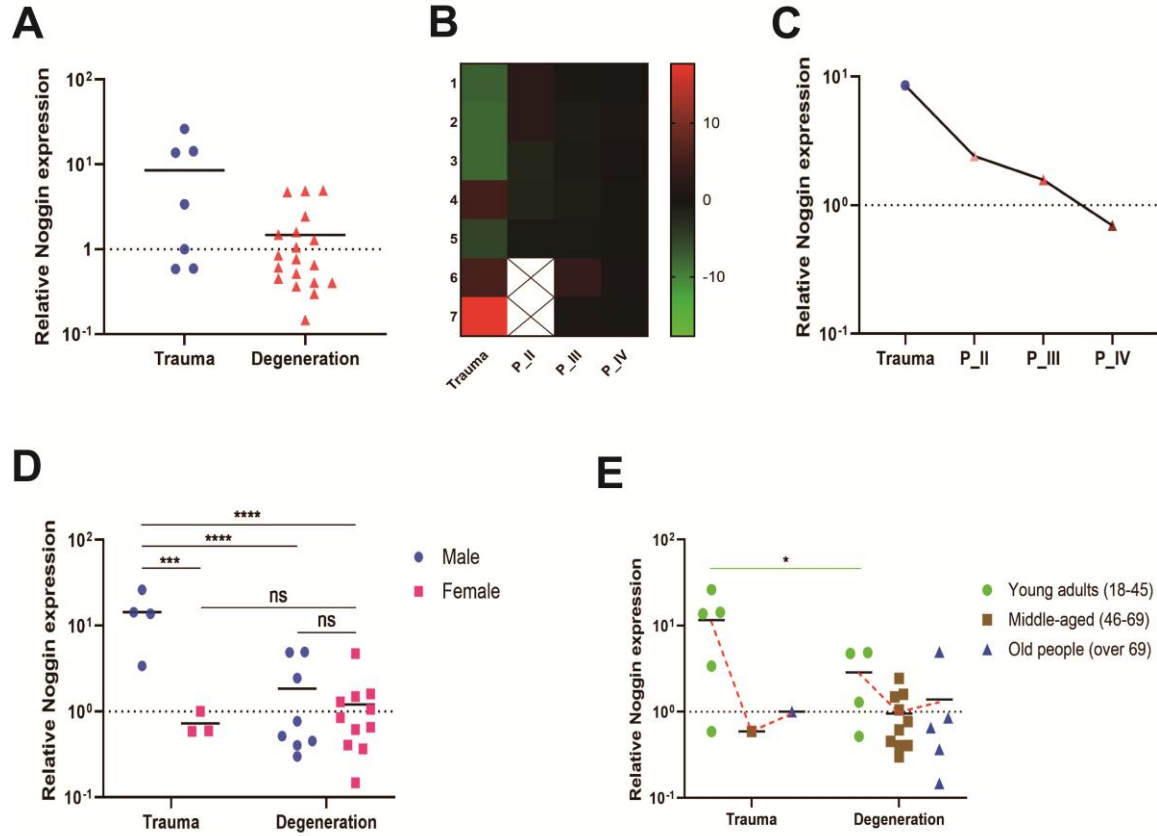
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Abstract

Low back pain (LBP) is a health issue that leads to considerable disability and socioeconomic burdens. The degeneration of the human intervertebral disc (IVD) is a key factor in the pathogenesis of LBP. During disc degeneration, the nucleus pulposus (NP), a critical component of IVD, undergoes significant alterations in structural composition and function. Recent studies have highlighted the importance of various genes in IVD health. Noggin has been described as an essential gene in nucleus pulposus cells (NPCs) and is known as a central inhibitor of osteogenesis in the bone morphogenic protein (BMP) pathway. In this study, we conducted a comparative analysis of Noggin expression in human NPCs from degenerative and healthy IVD. Twenty-six IVD samples (nineteen from degeneration and seven from trauma) were collected to separate NPCs for RNA extraction. Noggin expression was quantified at the transcript level by qPCR to analyze the difference between degenerative and healthy IVD. Results indicated that Noggin expression was lower in NPCs from degenerated IVD compared to trauma cases. The severity of IVD degeneration correlated with decreased Noggin levels. Healthy males exhibited higher Noggin expression than healthy females and those with IVD degeneration. Noggin expression peaked in young adults (18-45y), decreased in middle age (46-69y), and rose again in the elderly (over 69y), but remained lower than in youth. Furthermore, upper lumbar discs (T12/L1-L2/3) showed higher Noggin levels than lower lumbar discs (L3/4-L5/S1). This research elucidates valuable insights into the variability of Noggin concerning sex, age, degree of disc degeneration and disc location. It provides implications for IVD health and LBP treatment. Future research needs to confirm these insights at the protein level.

Key words. Low back pain, Human intervertebral disc, Nucleus pulposus cells, Noggin



Po3_1.08

Improved Intervertebral Disc Fusion with Biologics: The prostaglandin E2 receptor 4 agonist KMN159 acts synergistically with BMP2 and L51P to induce osteogenic differentiation of human annulus fibrosus cells

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Abstract

Introduction: Spinal fusion surgery is performed to restore proper spinal alignment, but it presents notable challenges, particularly in older patients with comorbidities. This study aims to identify factors that promote the fusion of intervertebral discs, focusing on minimally invasive approaches for spinal surgery. Specifically, we examine a novel method to induce osteogenic differentiation in annulus fibrosus cells (AFCs), using a combination of bone morphogenetic protein-2 (BMP2), L51P (a BMP2 analog), and KMN159 (a prostaglandin E2 receptor 4 agonist).

Materials and Methods: Primary human AFCs (n=11) were stimulated with BMP2, L51P, and KMN159 and cultured for 21 days. Cell viability was assessed to detect cytotoxic effects following stimulation. At 7, 14, and 21 days, osteogenesis was evaluated by measuring the transcription levels of bone-related genes: alkaline phosphatase (ALP), Runt-related transcription factor 2 (RUNX2), bone gamma-carboxyglutamate protein (BGLAP), secreted phosphoprotein 1 (SPP1), osterix (SP7), and type I collagen (COL1). In parallel, BMP antagonists such as Noggin, Gremlin 1, and Chordin were quantified using quantitative polymerase chain reaction (qPCR). On Day 14, ALP activity was measured at the protein level, and on Day 21, histological staining with alizarin red (ALZR) was performed to detect calcium deposits, indicating mineralization.

Results: KMN159 had no discernible impact on the cell viability of AFCs but demonstrated weaker osteogenic effects on AFCs compared to BMP2. Specifically, fold changes in the expression of osteogenic markers on Day 21 showed BMP2 had a stronger effect than KMN159 in promoting the expression of genes such as ALP (2.828 vs 2.516), RUNX2 (4.199 vs 0.863), BGLAP (2.035 vs 0.7028), SPP1 (6.407 vs 1.078), SP7 (42.20 vs 1.094), and COL1 (1.664 vs 0.9518). When KMN159 was combined with BMP2 and L51P, there was a

significant increase in ALP expression in AFCs ($P < 0.05$), suggesting enhanced osteogenic differentiation. This result was corroborated by increased ALP protein activity and intensified calcium deposition detected by ALZR staining. Although other bone-related markers didn't show statistically significant changes, an upward trend in expression was observed after 21 days. KMN159 had minimal impact on BMP antagonists (Noggin, Gremlin 1, and Chordin), indicating its minimal influence on their expression.

Conclusion: Our results suggest the combination of KMN159, BMP2, and L51P can successfully induce osteogenic differentiation in human AFCs. This combination may serve as a promising minimally invasive approach to enhance spinal fusion without removing the intervertebral disc, offering potential new treatment options for patients suffering from lower back pain.

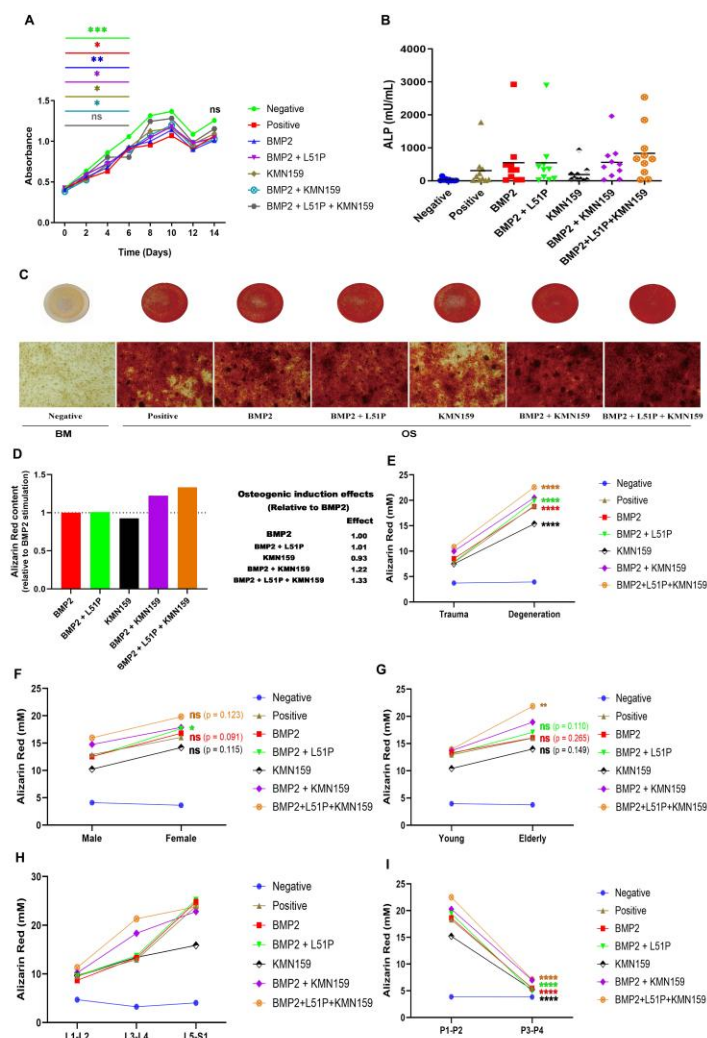


Figure: The combination of KMN159, BMP2 and L51P successfully induce osteogenic differentiation in human AFCs.

(A) KMN159 didn't affect the growth of AFCs.

(B) ALP assay showed that at the protein level, KMN159 combined with BMP2 and L51P could increase the expression of ALP compared with other groups.

(C) A trend of higher calcium deposit could be observed in BMP2+L51P+KMN159 group on Day 21 using alizarin red staining (Calcium deposits: Bright orange red).

(D) The osteogenic induction efficacy relative to BMP2 across different groups.

(E - I) The AFCs derived from the degenerative, female, elderly, lower lumbar and low passage discs were more effectively induced for osteogenesis.

(Shown as means. p-value, ns > 0.05; * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001; n = 4-11).

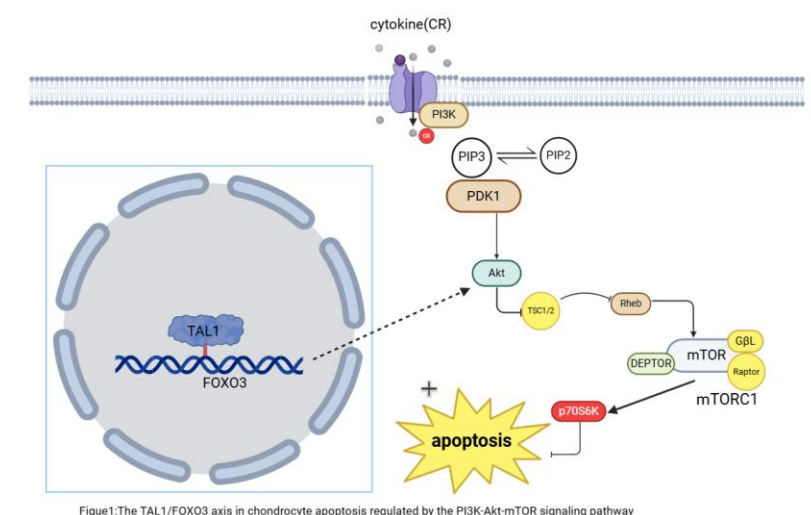
Po3_1.09

TAL1/FOXO3 axis in chondrocyte apoptosis regulated by the PI3K-Akt-mTOR signaling pathway in LFJOA

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Abstract



Osteoarthritis of lumbar facet joints (LFJOA) can cause intractable low back pain, intermittent claudication and other more severe symptoms associated with neurological defects, causing great economic burden on patients and society. Apoptosis of chondrocytes is one of crucial process. However, the genes that play a vital role in this process are still poorly studied. First, patients requiring lumbar posterior decompression surgery were stratified into control (Weishaupt grade 0-1) and degeneration (Weishaupt grade 2-3) groups based on standardized MRI classification. Whole-transcriptome sequencing of facet joint articular cartilage revealed significant downregulation of *TAL1* expression and dysregulation of the PI3K-Akt-mTOR pathway in the degeneration group. Through CRISPR/Cas9-mediated gene editing in primary chondrocytes, we established *TAL1* overexpression and knockdown models, with subsequent validation of apoptotic markers and pathway activity via RT-qPCR and Western blotting. In vivo, intra-articular injection of AAV9-*TAL1* following monoiodoacetate (MIA)-induced lumbar facet joint osteoarthritis (LFJOA) in rats demonstrated chondroprotective effects. Bioinformatics analysis using ChipBase predicted *FOXO3* as a downstream target, which was confirmed by chromatin immunoprecipitation (ChIP) assay. Mechanistically, *TAL1* transcriptionally regulates

FOXO3 to modulate PI3K-Akt-mTOR signaling and suppress chondrocyte apoptosis. This study highlights the important role of the TAL1/FOXO3 axis in chondrocyte apoptosis regulated by the PI3K-Akt-mTOR signaling pathway in the progression of LFJOA. Inhibition of chondrocyte apoptosis by activation of the TAL1/ FOXO3 axis is expected to be a novel target for the treatment of LFJOA.

Po3_1.10

ECM degradation-driven accumulation of cytotoxic V δ 1 T-cells in Modic type 1 changes

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Abstract

INTRODUCTION: Modic type 1 changes (MC1) are associated with chronic low back pain (cLBP). Despite many studies pointing to the role of inflammation in MC1, a comprehensive investigation of the immune system in MC1 lesions is missing.

OBJECTIVE: To investigate the immunological background of MC1 in relation to degeneration of the adjacent intervertebral disc.

METHODS: Vertebral bone marrow aspirates were collected from cLBP patients undergoing lumbar spinal fusion, and mononuclear cells were isolated. Cells (n=22, MC1+intra-patient control=8+8, control patients=6) were immunophenotyped by massively parallel flow cytometry. T-cell functional properties were analyzed by flow cytometry: 1.) ex vivo analysis of Granzyme B, 2.) Cytokine release after in vitro stimulation with Brefeldin and PMA/ionomycin, 3.) Proliferation in response to IL-15 followed by decreased CFSE fluorescence. Single-cell RNA-sequencing: MC1 and control biopsies (n=4+4) were enzymatically digested, neutrophils depleted, and ~10'000 cells sequenced. Extracellular matrix protein analysis: Intervertebral discs were analyzed by LC-MS/MS. Cartilage oligomeric matrix protein (COMP) was quantified in bone marrow plasma with ELISA. COMP functional assay: bone marrow-derived mononuclear cells were treated with COMP, and cytokine production was analyzed using flow cytometry.

RESULTS: Immunophenotyping revealed accumulation of V δ 1 T-cells in MC1 (Fig.1A,B) and dysregulated expression of surface markers on V δ 1 T-cells in MC1 (Fig.1C). Most V δ 1 T-cells in MC1 expressed NKp80 (72% vs. 40% in intrapatient control) and NKp80 was more expressed on V δ 1 T-cells than on other T-cells (Fig.1D). NKp80⁺ V δ 1 cells expressed more Granzyme B and, upon stimulation, produced more IFN γ and TNF than other T-cells (Fig.1E). V δ 1 T-cells also expressed more IL15R α than other T-cells (Fig. 1F), and IL-15 selectively induced their proliferation (Fig.1G). IL-15 levels in bone marrow plasma correlated with V δ 1 frequency (R=0.84). Single-cell RNA-sequencing revealed that IL15 was expressed predominantly in CD16⁺ monocytes, and that CD16⁺ monocytes accumulated in MC1

(Fig.1H). COMP-activated CD16+ monocytes in-vitro. COMP was enriched in discs adjacent to MC1 and in MC1 bone marrow (Fig.1I,J,K).

CONCLUSION: We discovered an accumulation of cytotoxic and pro-inflammatory V δ 1 T-cells in MC1 lesions that might be linked to degeneration of the adjacent intervertebral disc. Their proliferation was closely related to marrow levels of IL-15, and they represented the only T-cell subset that proliferated in response to IL-15. A subset of CD16+ monocytes accumulating in MC1 was discovered as the primary source of IL-15 in MC1, and we revealed that they can be activated by disc matrix-derived protein COMP, which also accumulates in MC1.

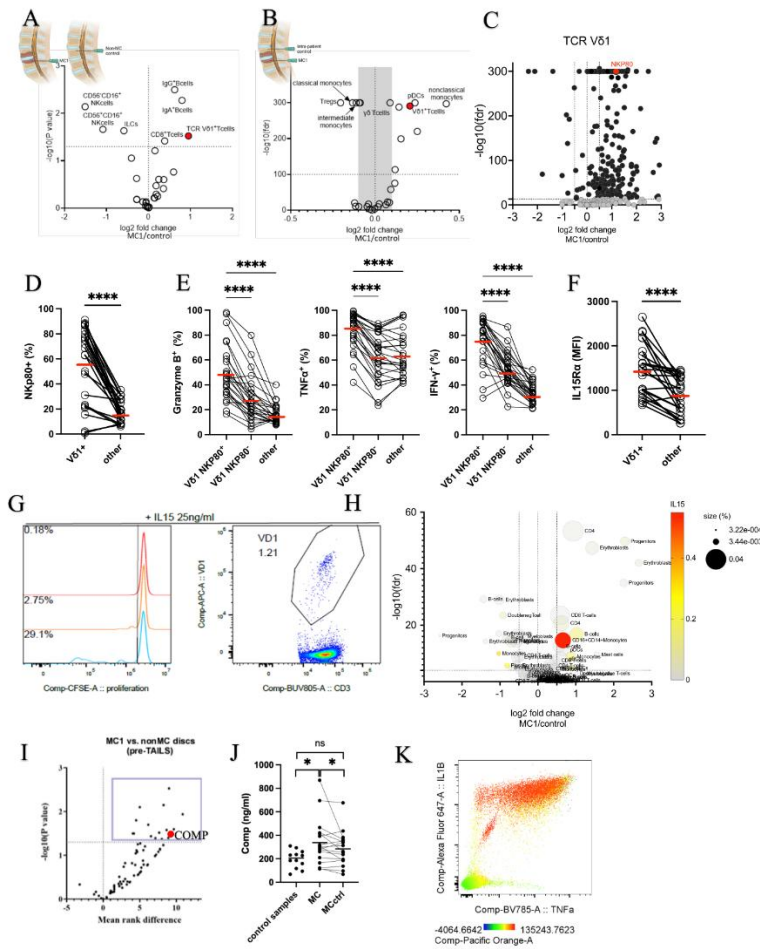


Figure 1. A.) Comparison of immune cell subset presence in MC1 patients and patients without MC1 based on flow cytometry screening of bone marrow aspirates. B.) Comparison of immune cell subset frequencies in MC1 lesions and intrapatient controls based on flow cytometry screening of bone marrow aspirates. C.) Changes in the expression of surface markers on T-cells from MC1 and control tissue. Markers mentioned in the text are highlighted in red. D.) Comparison of NKp80 expression between T cell subsets. E.) Comparison of Granzyme B, IFN γ , and TNF α expression between NKp80 $^{+}$ V β 1 T-cells and other T-cells. F.) Comparison of CD215 expression between T cell subsets. MFI=Median Fluorescence Intensity G.) The proliferation of CD4 $^{+}$ $\alpha\beta$ T-cells, CD8 $^{+}$ $\alpha\beta$ T-cells, and V β 1 T-cells in response to IL15. H.) Comparison of clusters of immune cell presence in MC1 lesions and intrapatient control based on single-cell RNA seq of bone marrow biopsies. Expression of IL15 RNA is shown as color code. I.) Comparison of protein presence in intervertebral discs of MC1 and control patients using mass spectrometry. J.) Comparison of COMP levels in bone marrow plasma in MC1 lesions, intrapatient controls, and control patients by ELISA. K.) Activation of PBMcs by 20ug/ml of COMP. Expression of TNF α , IL1 β , and IL14 was followed by flow cytometry. The expression of CD14 is depicted as a color code.

Po3_1.13

Immune cell alterations in patients with post-operative infections of the spine

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Abstract

Surgical site infections (SSIs) are a significant complication that can occur after spine surgery. They are associated with extended hospitalizations, higher morbidity, increased rates of re-operation, and, in some cases, a greater risk of mortality. This study examined changes in immune cell profiles in the blood of patients who developed infections after spinal surgery (Infection group - I, $n=17$) compared to control patients without infections (Non-infection - NI, $n=20$). The two groups were matched by age, body mass index (BMI), and the invasiveness of the surgical procedure. Peripheral blood mononuclear cells (PBMCs) from the two patient groups were collected and subjected to high-dimensional mass cytometry, enabling detailed immunophenotyping of various immune cell subsets. The analyses included adaptive immune cells such as T cells and B cells, as well as innate immune populations such as dendritic cells as well as activation and exhaustion markers for CD4+ and CD8+ T cells.

A cluster analysis identified 46 immune cell clusters, 30 of which exhibited significant differences between the I and NI patients. Of these 30 clusters, six clusters were upregulated and 24 downregulated in I patients compared to NI patients. Th17 and B cells were significantly increased in I patients compared to NI patients ($p=0.0152$ and $p=0.0305$ respectively). CD4+ T cells showed no differences between groups, but activation marker CD69 and HLA-DR were increased in I compared to NI patients ($p=0.0023$; $p=0.0032$). Double positive and gamma delta T cells, as well as innate immune cells such as NK cells and monocytes were decreased in I patients compared to NI patients ($p=0.0305$; $p=0.0070$; $p=0.0043$; $p=0.0357$). Moreover, myeloid and plasmacytoid dendritic cells were decreased in I patients compared to NI patients ($p=0.0011$ and $p=0.0458$, respectively).

This study highlights that patients with a spine-related infection exhibit an altered immune cell profile characterized by increased B cell numbers, and decreased numbers of innate immune cells. Further investigation into activation and exhaustion markers may reveal deeper mechanisms of immune response in these patients.

Po3_1.14

Mitophagy inhibitor SPP1 regulates nucleus pulposus cell fate during degeneration and calcification by activating ITG α 5/ β 1 and inhibiting ubiquitin-dependent PINK1/PARKIN pathway

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Abstract

Low back pain (LBP) caused by nucleus pulposus degeneration and calcification leads to great economic and social burden worldwide. Unexpectedly, no previous studies have demonstrated the association and the underlying mechanism between nucleus pulposus tissue degeneration and calcification formation. Secreted Phosphoprotein 1 (SPP1) exerts crucial functions in bone matrix mineralization and calcium deposition. Here, we report a novel function of SPP1, namely that it can aggravate nucleus pulposus cells (NPs) degeneration by negatively regulating extracellular matrix homeostasis. The degenerated NPs have a higher mineralization potential, which is achieved by SPP1. Mechanistically, SPP1 can accelerate the degeneration of nucleus pulposus cells by activating integrin receptor α 5 β 1 (ITG α 5/ β 1), aggravating mitochondrial damage and inhibiting mitophagy. SPP1-ITG α 5/ β 1 axis inhibits mitophagy by PINK1/PARKIN pathway blockade. In conclusion, SPP1 activates ITG α 5/ β 1 to inhibit mitophagy, accelerates NPs degeneration and induces calcification, thereby leading to intervertebral disc degeneration (IVDD) and calcification, identifying the potentially unknown mechanism and relationship between IVDD and calcification. We provide important insights into the role of SPP1 in nucleus pulposus calcification in IVDD by inducing nucleus pulposus cell senescence through inhibition of mitophagy and may help develop potential new strategies for IVDD treatment.

Po3_1.17

Plasmacytoid dendritic cells expand in Modic type 1 change bone marrow lesions and overexpress toll-like receptor 4

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Abstract

INTRODUCTION: Bone marrow (BM) inflammation is a hallmark of painful vertebral BM lesions called Modic type 1 changes (MC1). Bulk RNA sequencing has revealed upregulated neutrophil, monocyte, T-cell, and B-cell signatures in MC1 BM cells. However, the key cellular players underlying MC1 inflammatory processes remain poorly understood, limiting disease-modifying treatment development. The aim of this study was to identify pathomechanistically relevant MC1 cell populations using single-cell RNA sequencing (scRNAseq) and massively parallel flow cytometry, and to characterize their cytokine response to pro-inflammatory stimulation.

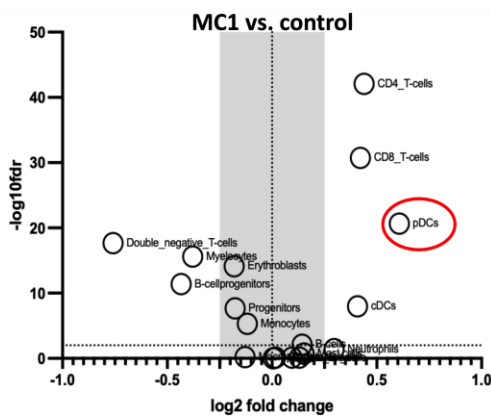
METHODS: MC1 and intra-patient control BM samples were collected from cLBP patients with MC1 undergoing spinal fusion. scRNAseq: Neutrophil-depleted single-cell suspensions (MC1: n=4, control: n=4) were sequenced (10x Genomics; 10,000 cells/sample). Cellular fractions were compared using a generalized linear mixed-effects model, differentially expressed genes ($p < 0.05$) were identified, and overrepresentation analysis was performed. Flow cytometry: Mononuclear cells (n=8+8) were analyzed using massively parallel flow cytometry (360 markers, CYTEK Aurora). Cellular fractions and surface protein expression were compared using a generalized linear mixed-effects model. Additionally, cytokine expression after 4h LPS stimulation was assessed with flow cytometry.

RESULTS: By scRNAseq, we found a +55% fractional increase in plasmacytoid dendritic cells (pDCs) in MC1 BM (MC1: $3.73 \pm 1.05\%$, control: $2.40 \pm 0.73\%$, false discovery rate (FDR) < 0.001) (**Figure 1A**). Flow cytometry confirmed a similar trend, with pDCs among the top three increased cell types in MC1 (MC1: $3.10 \pm 1.00\%$, control: $2.60 \pm 0.86\%$, MC1 vs. control: +19%, FDR < 0.001). Toll-like receptor 4 (TLR4) was one of the most upregulated proteins in MC1 pDCs (**Figure 1B**). Despite this, pro-inflammatory cytokine expression (interferon (IFN)-alpha (IFN- α), IFN- β , IFN- γ , interleukin (IL)-2 (IL-2), IL-5, IL-10, IL-17, tumor

necrosis factor alpha (TNF- α) after LPS (TLR4 agonist) stimulation did not differ between MC1 and control pDCs. Transcriptomic analysis revealed upregulated T-cell activation (FDR<0.001) and response to purine-containing compound (FDR<0.001) in MC1 pDCs.

DISCUSSION: We identified pDCs as key regulators of MC1 inflammatory processes. MC1 pDCs might expand and become activated via the TLR4-agonist neutrophil elastase (NE). MC1 BM neutrophils release higher NE levels, a key component of purine-containing neutrophil extracellular traps, aligning with the MC1 pDC transcriptomic signature. The TLR4-agonist origin remains to be elucidated, but chronic TLR4-agonist exposure is suggested by the reduced responsiveness of MC1 pDCs after LPS stimulation. The transcriptome also indicates T-cell activation, suggesting pDCs may drive the previously reported T-cell enrichment. Thus, pDCs are potential disease-modifying targets to reduce BM inflammation in MC1.

A) Plasmacytoid dendritic cells (pDCs) accumulate in MC1 bone marrow



B) MC1 pDCs overexpress TLR4

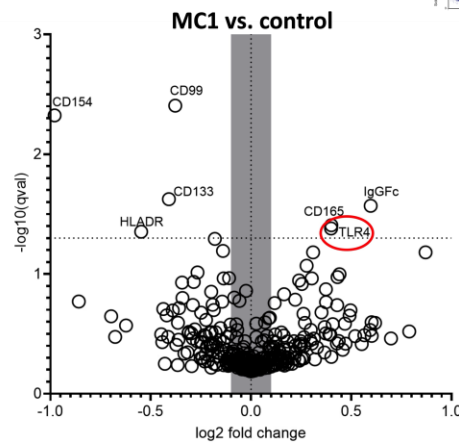


Figure 1A) Cell fractions identified by scRNAseq. **B)** Differentially expressed proteins (flow cytometry)

Po3_1.18

Impact of Caudal Segment Bone Fusion on Rod Load at the Cranial Segment in a Two-Level Spinal Interbody Fusion Construct – A Biomechanical Study

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Abstract

Purpose: One-dimensional implant load has been validated as a method for assessing spinal fusion progression in preclinical and in vitro studies. However, multilevel fusion procedures, which are more susceptible to non-union and implant failure than single-level fusions, pose additional challenges. Specifically, the need for implantable sensors at each segment is questioned, especially in space-constrained regions like L5-S1, where non-union and implant failure are more common. This study explores whether a single sensor at the cranial level can effectively monitor fusion progression of a caudal segment in a two-segment posterior instrumentation following TLIF surgery. It is hypothesized that adjacent level fusion has no influence on the rod load above the separating pedicle screw.

Methods: Seven human cadaveric spines, stabilized with posterior instrumentation following TLIF surgery at L4-L5 and L5-S1, were used. Bone fusion was already simulated at L4-L5, whereas level L5-S1 was left with the TLIF instrumentation for the initial test and bone fusion was simulated for the subsequent test. Flexibility testing and implant load measurements were conducted to compare segmental range of motion (ROM), and rod strain measured at L4-L5 between states of unfused and simulated fusion of L5-S1.

Results: Axial strain measurements at L4-L5 during flexion-extension (FE), lateral bending (LB), and axial rotation (AR) were unaffected by L5-S1 fusion. However, torsional strain at L4-L5 decreased during AR following L5-S1 fusion. A strong correlation was found between the relative decrease in ROM and torsional strain during AR motion.

Conclusion: Implantable strain sensors currently used in research to investigate fusion progression are unaffected by adjacent level fusion. The decrease in implant load measured by those sensors, therefore, only reflects biomechanical changes at the segment containing the sensor. Although a strong correlation between the reduction in AR-ROM and AR-torsional strain was observed after fusion of the adjacent segment, torsional strain measurement is not viable for clinical use due to the expected low signal in vivo and the inability to distinguish between the segments contributing to the changes in implant load.

Po3_1.20

Is there an association between scoliosis in childhood and adolescence and long term risk of cancer? A systematic review.

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Abstract

Introduction. Adolescent Idiopathic Scoliosis (AIS) affect teenagers, during their final growth and creates coronal deformity of the spine. The prevalence exceeds 1%; the etiology is believed to involve genetic and environmental factors. Diagnosis relies on radiographic evaluation, often resulting in substantial exposure to ionizing radiation, a known carcinogen. This review aims to evaluate the long-term cancer risks associated with AIS, focusing on the roles of genetic predispositions and radiation exposure.

Methods. A comprehensive systematic search was conducted on August 5, 2024, across PubMed, EMBASE, Scopus, Cochrane Libraries, and CINAHL, covering studies from 1947 onward. Human studies on patients with scoliosis diagnosed before age 20 were included. For cancer assessment, both risk, incidence and mortality were included. Studies were excluded, if they focused solely on congenital or secondary scoliosis or if the only cancer types assessed were basal cell carcinoma or cervix uteri cancer. Screening and quality assessment were conducted using Covidence software. The first author performed the initial screening, while the first and second authors conducted full-text assessments and quality assessment collaboratively. Discrepancies were resolved through discussion, yielding a final agreement score of 0.83.

Discussion. Seven studies from the U.S., Australia, Denmark and the Netherlands were identified. Notable findings included elevated breast cancer risks among U.S. cohorts, linked to historical radiographic practices that delivered higher radiation doses. Notable limitations among the studies included small sample sizes. Four of the seven studies were overlapping and derived from the U.S. Scoliosis Cohort. Variations in study designs and cancer outcome measures limited the feasibility of meta-analysis. None of the included studies assessed genetic etiologies of cancer. Risk of bias in the studies were generally attributed to selection bias and underreporting of characteristics and confounding variables. While most studies included either showed a tendency or a significant association towards scoliosis and risk of cancer, most findings were based on historical data with exposure of radiation several orders of magnitude larger than modern standards, both due to changes in projection from anteroposterior to posteroanterior, and due to reduction of use of ionizing radiation. Therefore, it is important to note, that these changes could be a major factor in risk of cancer identified in historical cohorts.

Conclusion. This review highlights the the importance of modern studies to asses the development of cancer among AIS patients, with modern radiological techniques. Further research in the cancer types in AIS patients should be conducted, to understand the causes.

Po3_1.22

How to implant a degradable, regenerative annulus fibrosus closure? An in vitro study.

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Abstract

Introduction. Disc herniations are defined as a displacement of disc material beyond the boundaries of the disc space [1]. With a lifetime risk of 1–3%, lumbar herniations represent a highly prevalent pathology [2]. A suitable closure technique could prevent re-herniation through the damaged annulus fibrosus (AF). The goal of this study was to evaluate a new implant made of a degradable scaffold of poly(ϵ -caprolactone-co-lactide) (Fig1A) that is inserted into the annulus defect to close the defect and to support new tissue formation [3]. The biomechanical performance of five different implantation strategies was compared.

Methods. Thirty bovine tail segments were assigned to five groups (n=6), prepared, and embedded in PMMA (Fig2). An AF defect was set in all groups; additionally, parts of the nucleus pulposus (NP) were removed in groups 2, 4, and 5. Then implants were inserted in all groups (Fig1B). In groups 3 and 4, a membrane glued to the AF additionally covered the implant (Fig1D). In group 5, the scaffold was implanted inversely and glued directly to the AF. The “extruded” part of the implant, directly pushed out by the intrinsic pressure or during loading, was measured after implantation and after each testing step. Implant failure was defined as migration >25% and then testing was aborted. Range of motion (ROM) was measured intact, implanted and after dynamic testing. Dynamic testing simulated four daily worst-case activities [4]. Statistics: Mann-Whitney-U ($p < 0.05$).

Results. The implants (length 6.8 ± 0.3 mm) could only be fully implanted when NP tissue was removed (Fig2). Except one, all implants of group 1–3 failed during the first flexibility test (Fig1CD). Implantation significantly increased ROM for groups 3, 4, and 5 ($p \leq 0.014$). 5 of 6 implants in group 4 and 4 of 6 in group 5 failed during the dynamic test simulating worst-case activities. None of the implants passed all tests.

Discussion. Our comparative study has assessed the primary stability of the regenerative scaffold implant with a newly developed test method already used for human specimens [4]. The study has shown an improved stability for implantation with a membrane seal or glue (groups 4/5). The study is limited by the different anatomy of bovine tail specimens, but the findings revealed new ideas for optimizing the implant. The development of a

regenerative AF closure is biomechanically essential to fully restore the herniated disc [5] and needs further insights from in vivo experiments.

Funding Fondation

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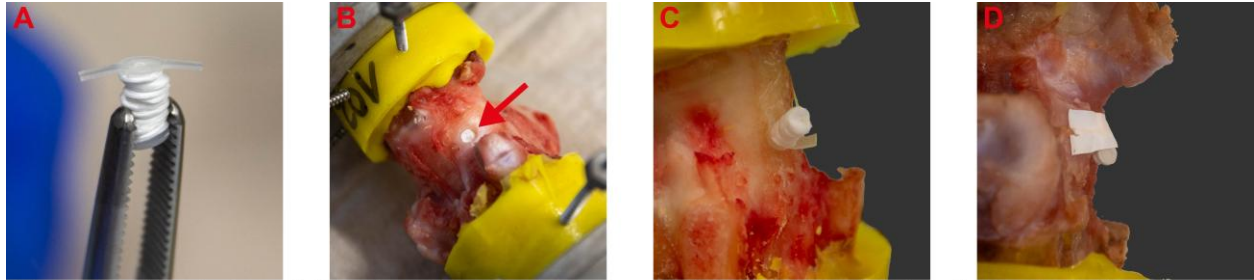


Figure 1: A) Implant consisting of a poly(ϵ -caprolactone-co-lactide) scaffold with wings for fixation in the AF defect. B) View of an example of an implanted scaffold in the AF of a bovine tail disc. The red arrow indicates the implant. C) Example of an extruded implant of group 1. D) Example of an extruded implant of group 2, which was additionally covered with a polytetrafluoroethylene membrane.

Po3_1.23

Development of High-throughput Assay to Screen Potential Drugs Identifies Papaverine as Neuroprotection Drug for Spinal Cord Injury via Blood-spinal Cord barrier protection

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Abstract

INTRODUCTION: Blood-spinal cord barrier (BSCB) protection can reduce secondary damage and promote recovery after spinal cord injury (SCI). This study aims to 1) develop a high-throughput screening assay (HTSA) to identify drugs protecting BSCB function and 2) screen 3,200 FDA-approved drugs to assess their therapeutic effects on SCI.

METHODS: Human brain endothelial cells (ECs) were cultured on 96-well plates with various hydrogen peroxide (H₂O₂) concentrations to optimize conditions for HTSA by measuring viability, cytotoxicity, and live/dead ratios. Using the optimized HTSA, 3,200 existing drugs were screened, and top candidate underwent to subsequent dose-dependency and oxygen-glucose deprivation (OGD) tests. A rat brain-derived in vitro BSCB model exposed to OGD stress with or without the candidate drug was assessed for trans-endothelial electrical resistance (TEER) and Na-F permeability. Next, C57BL/6 adult mice received intraperitoneal injections of the candidate drug or control, followed by SCI via wire-knife, with histological evaluation performed the next day. In a separate model, Lewis adult rats received C5 contusion injury, followed by 7 days of drug treatment, with histological and functional analysis conducted 8 weeks later. RNA sequence was used to explore the molecular mechanism of the candidate drug on human brain ECs.

RESULTS: The optimal H₂O₂ concentration for HTSA was 450 µM, yielding a Z'-factor of 0.75, S/B ratio of 2.9, and CV of 4%. Papaverine, a smooth muscle relaxant, emerged as the lead candidate, demonstrating protection against OGD stress in human ECs and preserving TEER and Na-F permeability (Fig 1). In vivo, Papaverine-treated subjects had reduced IgG leakage in mice, and reduced glial scar, cystic cavity volume, and improved gait and pain behaviors post-SCI in rats compared to controls (Fig 2). RNA sequencing suggested a novel mechanism underlying Papaverine's BSCB-protective effects.

DISCUSSION: The newly developed HTSA can successfully identify candidate drugs to protect human brain ECs from toxic stresses and identified Papaverine as a potential drug to protect BSCB functions from SCI. Though Papaverine is a known intracellular cAMP inducer, its protective effect on the BSCB appears to involve a distinct mechanism. As Papaverine is clinically applicable, it holds promise for translation into acute SCI therapy.

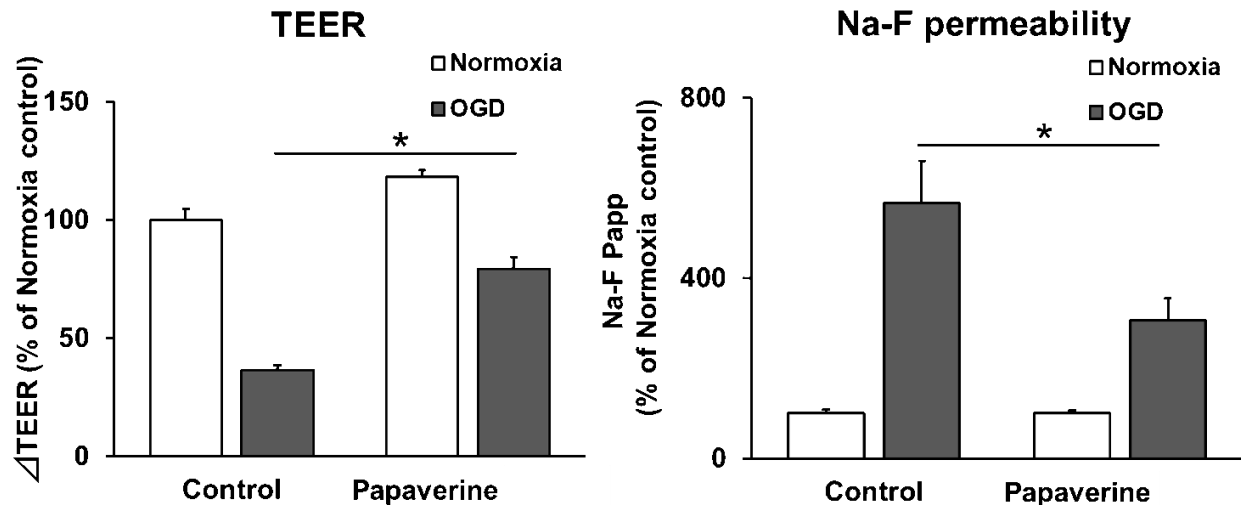


Fig.1 Papaverine protects BSCB function in vitro co-culture model using primary cells.

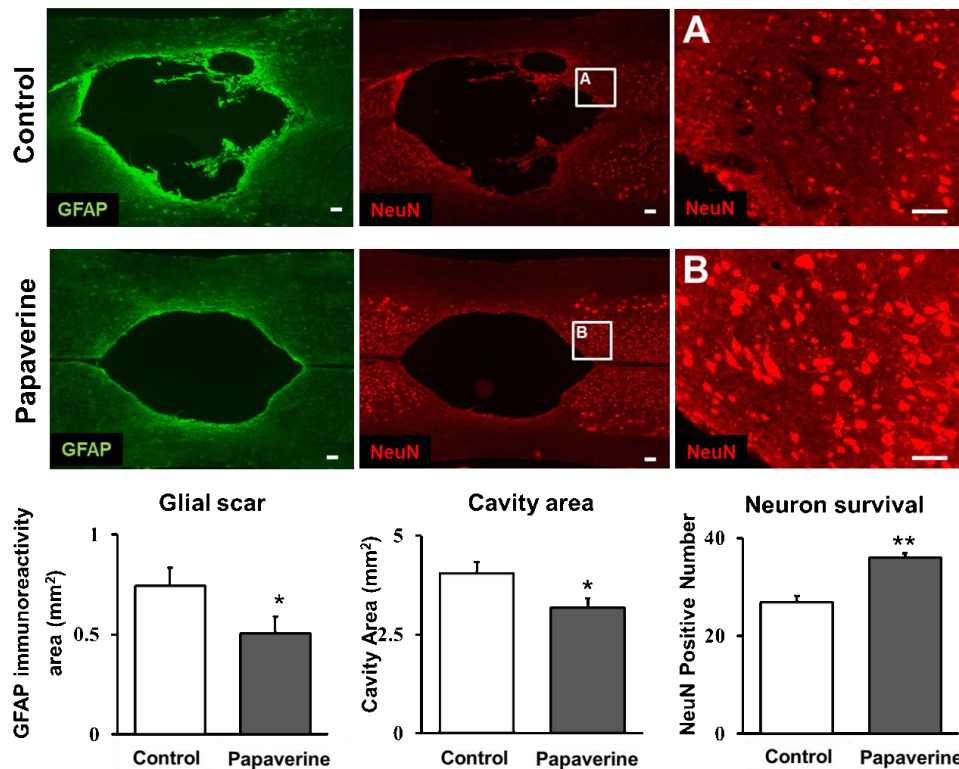


Fig.2 Papaverine has neuroprotective effect after SCI in rat.

Po3_1.25

3D bioprinting of whole intervertebral discs analogues reveals regulation of cell phenotype and regional matrix formation through stiffness and oxygen tension modulation

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Abstract

Introduction/Objectives. Intervertebral disc (IVD) degeneration is recognised as a leading cause of back pain and while studies have revealed roles for resident nucleus pulposus (NP) and annulus fibrosus (AF) cells in the degenerative process, tissue-engineered whole organ models are needed to better investigate the mechanisms governing tissue degeneration. This study therefore used microvalve-driven material jetting bioprinting to create four novel multi-material, whole IVD analogues and investigated the impact of regional matrix stiffness and oxygen tension on disc cell phenotype since these factors are closely associated with IVD degeneration.

Methods. Patient-derived primary human NP and AF cells were seeded into shear-thinning, low-viscosity, alginate-collagen hydrogels and patterned into a biphasic IVD structure via suspended layer additive manufacturing. Immunofluorescence staining was used to assess the nascent area, intensity, and integrated density of the phenotypic markers pro-collagen type I, collagen type VI, aggrecan, and hyaluronic acid produced in each bioprinted region following 21 days in culture.

Results. Differences in stiffness-mediated regulation of collagen and glycosaminoglycan production in AF cells were observed, and increased tissue stiffness upregulated collagen type VI production in NP cells but downregulated it in the AF. There was evidence that oxygen tension affected proteoglycan production, with hypoxia in particular increasing aggrecan and hyaluronic acid production in both cell types.

Conclusions. These IVD constructs represent the first whole IVD co-culture models created solely using bioprinting and expand the state-of-the-art in suspension bioprinted tissue analogues for regenerative research using regionally specific matrix cues matched to complex tissues. The findings provide novel insights into the importance of microenvironmental factors driving irreversible IVD degeneration.

Po3_1.28

Discordance in lumbar bone mineral density measurements by quantitative computed tomography and dual-energy X-ray absorptiometry in postmenopausal women: a prospective comparative study

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Shunde Hospital, Southern Medical University (The First People's Hospital of Shunde Foshan), Foshan, China

Abstract

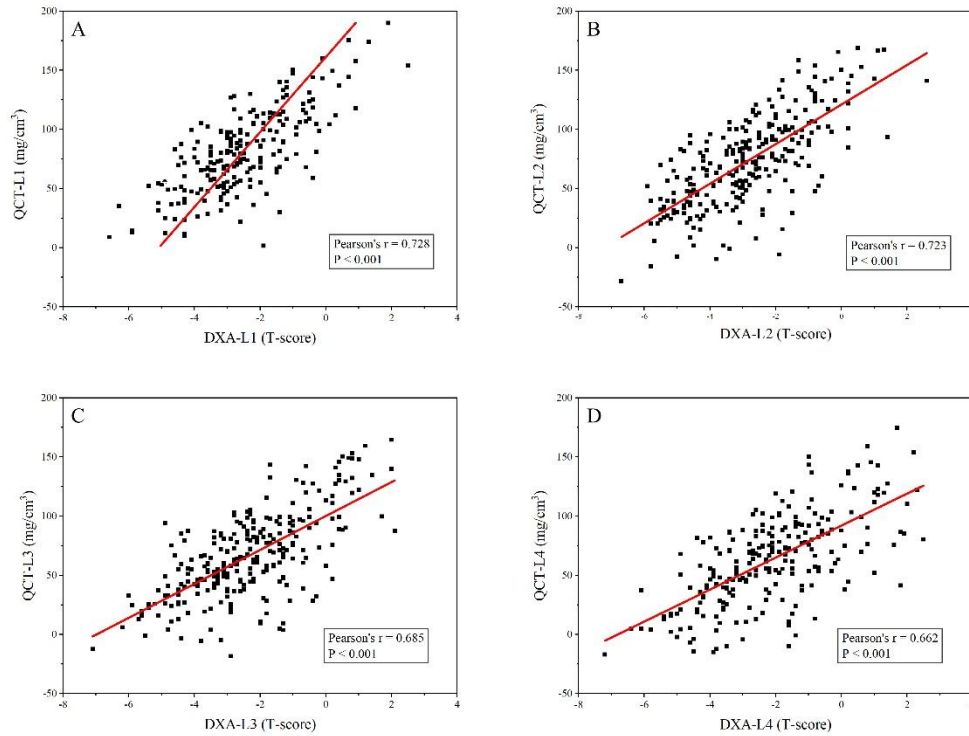
Background Context: Level-specific lumbar bone mineral density (BMD) evaluation of a single vertebral body can provide useful surgical planning and osteoporosis management information. Previous comparative studies have primarily focused on detecting spinal osteoporosis but not at specific levels.

Purpose: To compare the detection rate of lumbar osteoporosis between quantitative computed tomography (QCT) and dual-energy X-ray absorptiometry (DXA); to explore and analyze the distribution models of QCT-derived BMD and DXA T-score at the specific levels; and to evaluate the diagnostic accuracy of level-specific BMD thresholds for the prediction of osteoporotic vertebral compression fracture (OVCF).

Methods: Postmenopausal women who underwent QCT and DXA within a week of admission from May 2019 to June 2022 were enrolled. The diagnostic criteria for osteoporosis recommended by the World Health Organization and the American College of Radiology were used for lumbar osteoporotic diagnosis. Data between QCT and DXA were visualized in a stacked bar plot and analyzed. Correlations between DXA and QCT at the specific levels were evaluated using Pearson's linear correlation and scatter plots. Curve fitting of BMD distribution, receiver operating characteristic (ROC) and area under the curve (AUC) for each single vertebral level was performed.

Results: Of the 296 patients, QCT diagnosed 61.1% as osteoporosis, 30.4% as osteopenia and 8.4% as normal. Significant correlations in level-specific BMD between DXA and QCT were observed ($p < 0.001$), with Pearson's correlation coefficients ranging from 0.662 to 0.728. The correlation strength was in the order of $L1 > L2 > L3 > L4$. The low BMD detection rate for QCT was significantly higher than that for DXA at the L3 and L4 levels (65% vs. 47.9% and 68.1% vs 43.7, respectively, $p < 0.001$). Patients with OVCF showed significantly lower QCT-derived BMD (47.2 mg/cm³ versus 83.2 mg/cm³, $p < 0.001$) and T-score (-3.39 versus -1.98, $p < 0.001$) than those without OVCF. Among these patients, 82.8% (101/122) were diagnosed with osteoporosis by QCT measurement, while only 74.6% (91/122) were diagnosed by DXA. For discrimination between patients with and without OVCF, QCT-derived BMD showed better diagnosed performance (AUC range from 0.769 to 0.801) than DXA T-score (AUC range from 0.696 to 0.753).

Conclusion: QCT provided a more accurate evaluation of lumbar osteoporosis than DXA. The QCT-derived BMD measurements at a specific lumbar level have a high diagnostic performance for OVCF.



Scatter plot demonstrating a linear fit between QCT-derived and DXA T-score BMD measurements

Po3_1.29

Hypoplasia of the atlas pedicle in patients with os odontoideum: a CT-based morphometric analysis

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Abstract

Objective: To measure and compare radiographic parameters of the C1 between patients with/without os odontoideum (OsO) and to discuss their influence on surgical decision-making.

Methods: From January 2014 to June 2019, 50 patients with OsO (OsO group) and 50 with normal odontoids (control group) were consecutively included in the present study. Data, including demographic variables, clinical findings, surgical decision, and radiological features, were collected by retrospective chart review of patient records. Morphological changes were documented and anatomic parameters of the C1 measured from computed tomography scans. C1 pedicles were classified as type I (height of the C1 pedicle < 3.5 mm) or type II (height $\geq$ 3.5 mm) and the measurements obtained from the two groups compared.

Results: A total of 100 patients (49 men and 51 women) were deemed eligible for enrollment. Mean age of patients in the OsO group was 44.8 ± 12 years and that in the control group was 39.1 ± 14.5 years. C1 transverse diameter and distance from the midline to the inner edge of the transverse foramen in the OsO group were 14.91 ± 1.77 and 21.59 ± 1.8 mm, respectively, whereas those in the control group were 14.37 ± 1.18 and 22.15 ± 1.99 mm, respectively ($p < 0.05$). Radiographic parameters of the C1 pedicle, including width, medullary cavity height, and height, differed significantly between patients with OsO and controls (5.48 vs. 7.34, 0.54 vs. 1.39, and 3.09 vs. 4.04 mm, respectively, $p < 0.001$). Further, the proportion of type I C1 pedicle in the OsO group was significantly higher than that in the control group (59% vs. 26%, $p < 0.001$). Of 26 patients with bilateral type I C1 pedicle in the OsO group, 16 (61.5%) underwent a TARP procedure, while 7 (26.9%) received C1–C2 pedicle screw. All patients with bilateral type II C1 pedicle received C1–C2 pedicle screw fixation.

Conclusions: OsO may be associated with other abnormal anatomical features and morphology of the atlas. The results of the current study reveal that C1 pedicle size is smaller in patients with OsO than in those without OsO.

Po3_1.30

Assessment of bone density using the 1.5 T or 3.0 T MRI-based vertebral bone quality score in older patients undergoing spine surgery: Does field strength matter?

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Abstract

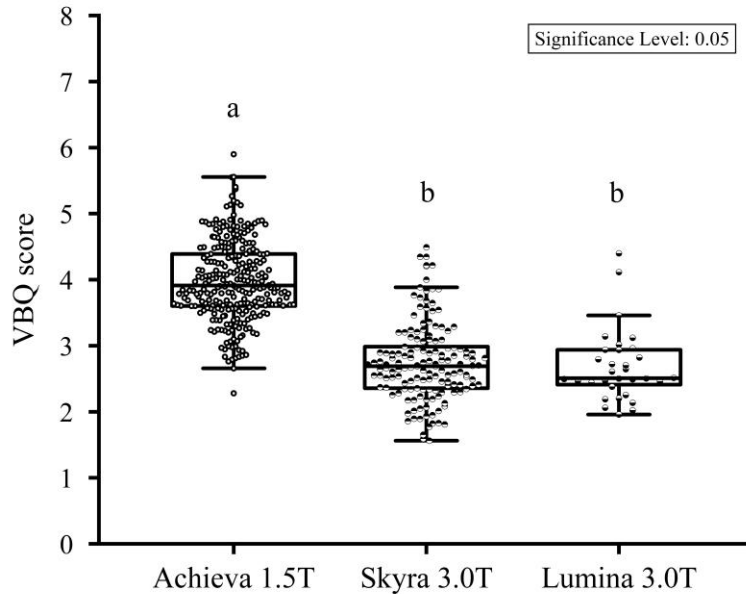
Background context: Recently published studies have revealed a correlation between MRI-based vertebral bone quality (VBQ) score and bone mineral density (BMD) measured using dual X-ray absorptiometry (DXA) or quantitative computed tomography (QCT). However, no studies have determined if differences in field strength (1.5 vs. 3.0 T) could affect the comparability of the VBQ score among different individuals.

Purpose: To compare the VBQ score obtained from 1.5 T and 3.0 T MRI (VBQ_{1.5T} versus VBQ_{3.0T}) in patients undergoing spine surgery and assess the predictive performance of VBQ for osteoporosis and osteoporotic vertebral fracture (VCF).

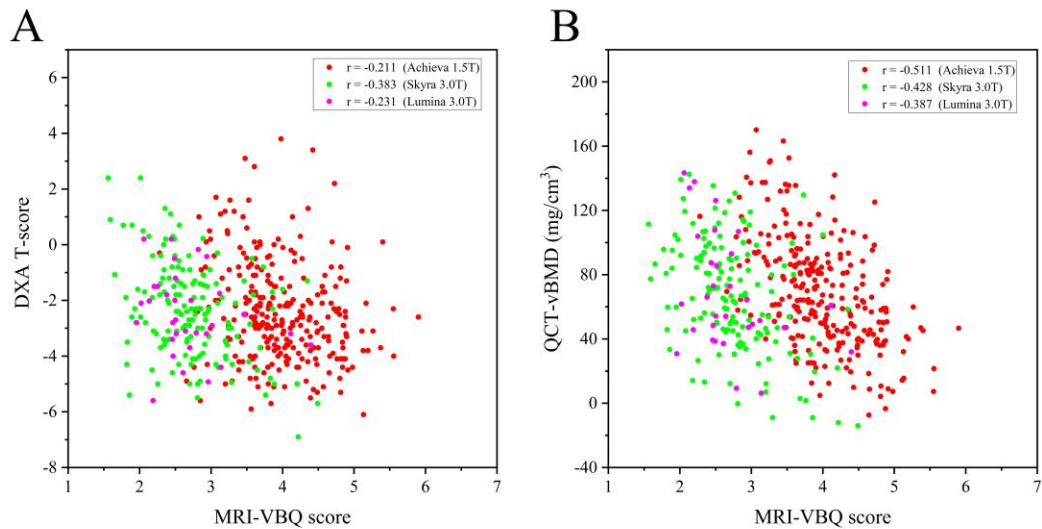
Methods: The osteoporotic classifications recommended by the World Health Organization and American College of Radiology were used to categorize the DXA T-score and QCT-derived BMD, respectively. For each patient, the VBQ score was calculated using T1-weighted MR images. Correlation analysis between VBQ and DXA/QCT was performed. Receiver operating characteristic (ROC) curve analysis, including determination of the area under the curve (AUC), was performed to assess the predictive performance of VBQ for osteoporosis.

Results: A total of 452 patients (98 men aged > 60 years and 354 postmenopausal women) were included in the analysis. Across different BMD categories, the correlation coefficients between the VBQ score and BMD ranged from -0.211 to -0.511, and the VBQ_{1.5T} score and QCT BMD demonstrated the strongest correlation. The VBQ score was a significant classifier of osteoporosis detected by either DXA or QCT, with VBQ_{1.5T} showing the highest discriminative power for QCT-osteoporosis (AUC = 0.744, 95% CI = 0.685-0.803). In ROC analysis, the VBQ_{1.5T} threshold values ranged from 3.705 to 3.835 with a sensitivity between 48% and 55.6% and a specificity between 70.8% and 74.8%, while the VBQ_{3.0T} threshold values ranged from 2.59 to 2.605 with a sensitivity between 57.6% and 67.1% and a specificity between 67.8% and 69.7%.

Conclusions: VBQ_{1.5T} exhibited better discriminability between patients with and without osteoporosis than VBQ_{3.0T}. Considering the nonnegligible difference in osteoporosis diagnosis threshold values between the VBQ_{1.5T} and VBQ_{3.0T} scores, it is essential to clearly distinguish the magnetic field strength when assessing the VBQ score.



Distribution of the vertebral bone quality (VBQ) score classified by different MRI scanners (Achieva 1.5T, Skyra 3.0T, and Lumina 3.0T).



Correlation analysis of MRI-based VBQ score versus DXA T-score (A, y-axis) and QCT-vBMD (B) for different MRI scanner types and manufacturers.

Po3_1.34

Calcium pyrophosphate dehydrate crystals induce pro-inflammatory and proteolytic gene expression in disc cells and are walled-off in situ

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Abstract

INTRODUCTION: Calcium pyrophosphate dihydrate (CPPD) crystals are associated with joint diseases like pseudogout but have received little attention in intervertebral discs. Despite their historical identification in disc tissues, their potential pro-inflammatory effects on disc cells remain unexplored. This study aimed to 1) analyze the relationship between CPPD crystals in discs and patient demographics, 2) assess their pro-inflammatory effects on disc cells in vitro, and 3) examine crystal localization in the disc, comparing in vitro findings within situ conditions.

METHODS: Histological analysis of 67 discs from 64 patients undergoing lumbar fusion was conducted using H&E, Masson trichrome, and non-aqueous alcoholic eosin (NAE). Patient demographics, pain scores and medical history were compared based on CPPD crystal presence. Statistical analysis included the Welch two-Sample t-test, Wilcoxon rank sum test, Fisher's exact test, and Chi-squared test. Disc cells were cultured primed with LPS for 24h and then exposed to CPPD crystals (50 µg/mL, 100 µg/mL) for 24 and 48 hours. Gene expression of inflammatory markers (IL6, IL8, CCL2) and catabolic enzymes (ADAMTS5, MMP1, MMP3, MMP13) was assessed via qPCR, with two-way ANOVA and Tukey-HSD post-hoc analysis used for statistical evaluation.

RESULTS: Dense CPPD crystal clusters were identified in 21% of discs and 20% of patients, with surrounding collagen condensation (Figure 1, arrows). Patients with CPPD crystals were older ($p=0.001$) and showed trends toward prior herniation ($p=0.082$) and surgery ($p=0.067$), but there was no correlation with the disability index (Figure 1A). Notably, 57% of MC1 discs contained CPPD crystals, compared to 21% in MC2 and 22% in non-MC discs (Figure 1B).

In vitro, CPPD crystals increased IL-1 β expression at 24 hours and IL6, MMP1, MMP3, and MMP13 at 48 hours, while ADAMTS5 and CCL2 levels remained unchanged (Figure 2).

DISCUSSION: Although CPPD crystals correlate with age, herniation, and prior surgeries, and elicit an inflammatory response in vitro, they appear unlikely to contribute to chronic low back pain through inflammatory mechanisms in situ. Once walled off, CPPD crystals are

isolated from surrounding tissue, potentially limiting their ongoing interaction with cells. Thus, their contribution to inflammation in degenerated discs may be episodic.

SIGNIFICANCE: This study demonstrates that CPPD crystals can induce inflammation in the disc when in direct contact with disc cells. It lays the foundation for future experiments exploring the temporal effects of CPPD exposure and the encapsulation process.

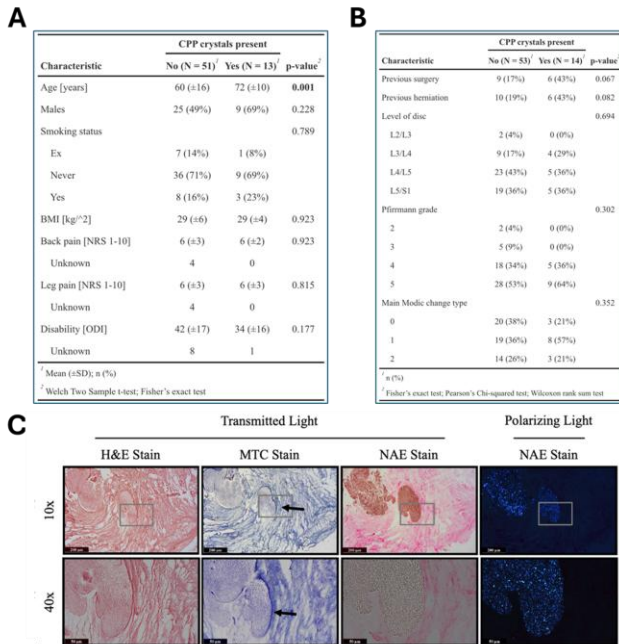


Figure 1. (A) Descriptive characteristics of the patient groups (NRS = numeric rating scale, ODI = Oswestry Disability Index). (B) Descriptive characteristics of the disc groups with and without CPPD crystals present. (C) Representative images of disc tissues with CPPD crystals taken with a light microscope and a polarizing microscope at different magnifications as indicated. Arrows indicate collagen condensation around crystals. Columns represent different staining methods.

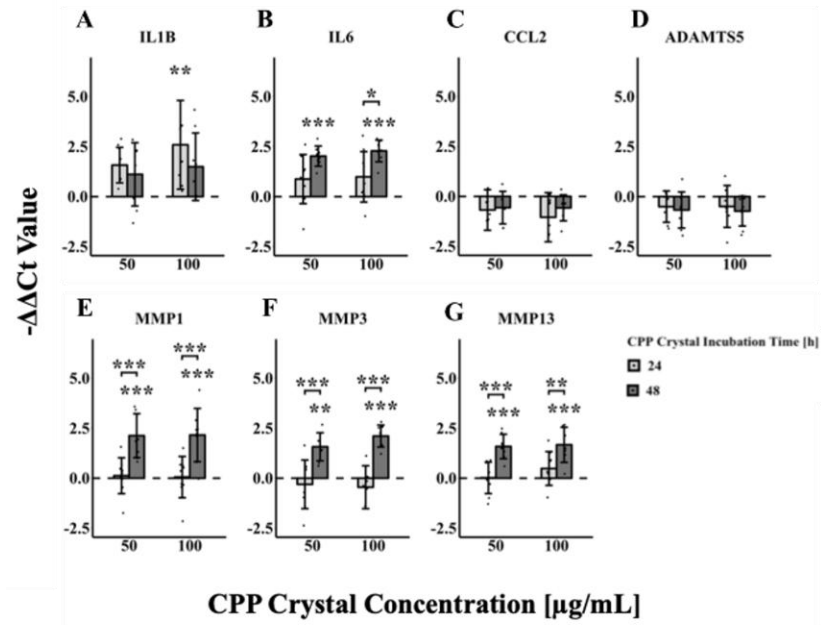


Figure 2. (A-G) Stimulation of disc cells for 24 and 48 hours with 50 ug/ml or 100 ug/ml of CPPD crystals. Gene expression of IL1 β , IL6, CCL2, ADAMTS5, MMP1, MMP3 and MMP13 was measured and are presented as bars representing log2 fold change relative to unstimulated cells, with standard deviation shown as error bars.

Po3_1.35

Intervertebral Disc State Fingerprint to classify degeneration and low back pain in the Northern Finland Birth Cohort 1966

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Abstract

INTRODUCTION. Intervertebral disc (IVD) degeneration (IDD) is a leading cause of low back pain (LBP), as biological and mechanical factors compromise disc integrity¹. Despite its prevalence, LBP's complex aetiology limits understanding, requiring methods to interpret heterogeneous data.

This study targets this challenge using the Disease State Fingerprint (DSF) technique², a visualization-decision-support tool to stratify and simplify biomedical data. We employed the Disease State Index (DSI)³ to compute multi-modal-variable alignments with a patient's disease state, e.g., biopsychosocial⁴ relationships with LBP-IDD.

METHODS. We analyzed 673 biopsychosocial variables from the Northern Finland Birth Cohort 1966 (NFBC1966), covering demographics (gender, daily-habits, economic-status, psychological-traits); laboratory results (cortisol, vitamins, blood-tests); musculoskeletal-assessments; anthropometrics; T2-weighted-MRI: Pfirrmann-grades, Modic changes, and radiomics. NFBC1966 comprises 5,525 individuals born in 1966⁵, assessed at 46-years.

Six models were developed: LBP-intensity and five for IDD at lumbar levels, two classes each (controls and positives). LBP-intensity (n=4531) was assessed using the Numerical Rating Scale (NRS 0–10), with NRS≥5 as positive. IDD (n=1,410) was classified using Pfirrmann-grades (positive≥3). Models used 80/20 training-testing split and 10-fold cross-validation.

RESULTS. Testing accuracy exceeded 70%, and LBP, L4-L5, and L5-S1 models achieved >80%, closely matching validation accuracy (Table.1).

Accuracy%	Pain-Sen.	L1L2	L2L3	L3L4	L4L5	L5S1
Validation	78.48	68.71	71.22	73.13	78.76	82.70
Testing	80.02	70.92	74.46	74.46	81.85	85.46

Table.1 Training-Accuracy

Fig.1A shows strong model performance (AUC>0.8) for LBP and IDD, particularly at lower lumbar levels. Fig.1B demonstrates DSI's ability to distinguish controls and positives.

Po3_1.37

The Role of Platelet-Rich Plasma (PRP) in Posterolateral Lumbar Fusion: A Meta-Analysis of Fusion Success and Safety With Subgroup Analyses by Instrumentation and PRP Preparation

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Abstract

Introduction: Posterolateral lumbar fusion (PLF) is a common surgical procedure for lumbar spinal disorders, but postoperative complications such as pseudarthrosis still have been challenging. As a source of growth factors, platelet-rich plasma (PRP) has been introduced as an adjunct to improve spinal fusion and reduce complications. However, evidence regarding its effectiveness and safety of PRP and whether the instrumentation type (instrumented vs. non-instrumented PLF) and PRP preparation methods (single-spin vs double-spin) influence these outcomes remains inconsistent.

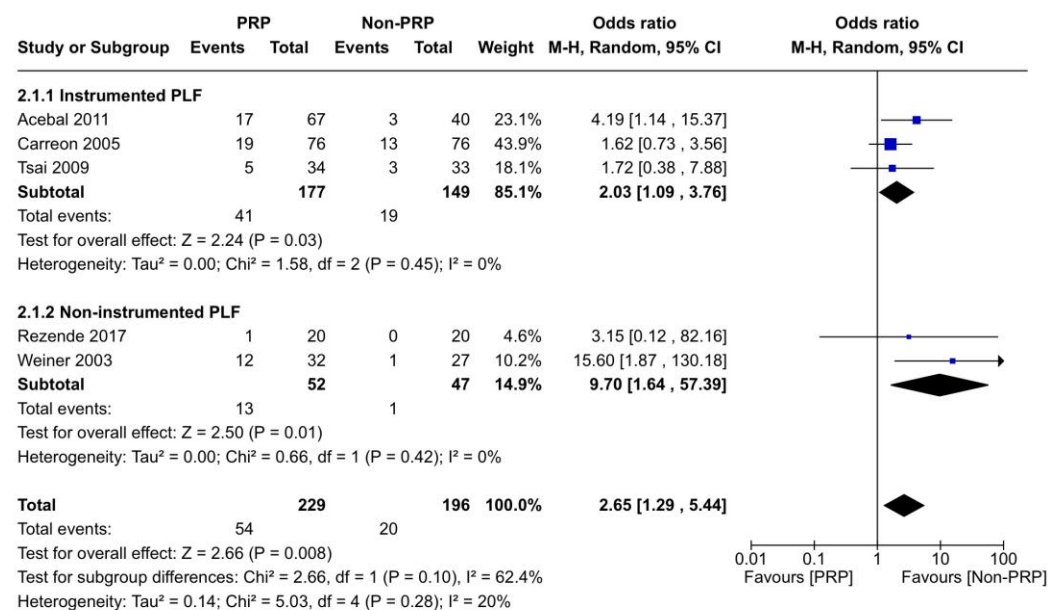
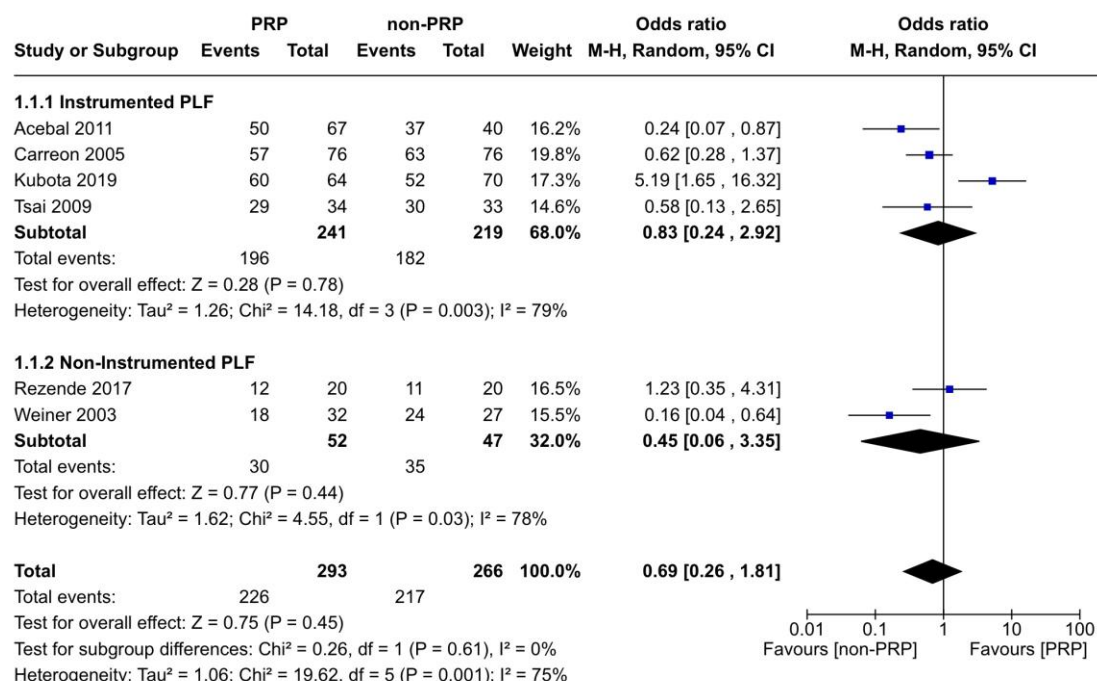
Objectives: This meta-analysis aims to compare the efficacy and safety of PRP in PLF and evaluate the impact of instrumentation type and PRP preparation methods through a pre-specified subgroup analyses.

Methods: Following PRISMA guidelines, a systematic search on PubMed, MEDLINE, and Scopus was conducted until January 2025. The primary outcome was fusion success (≥ 24 months follow-up), and secondary outcomes were pseudarthrosis, surgery revision, and graft resorption. These data were compared across instrumentation type and PRP preparation methods.

Results: Six studies (2 RCTs, 4 cohort studies) with a total of 487 patients were eligible. There was no significant difference in fusion success between PRP and non-PRP groups in both instrumented PLF (OR: 0.83 [0.24, 2.92]; $p = 0.78$) and non-instrumented PLF (OR: 0.45 [0.06, 3.35]; $p = 0.44$), with no statistical difference ($z = 0.51$) between the two instrumentation subgroups. Similarly, there was no difference between single-spin (OR: 0.75 [0.39, 1.47]; $p = 0.40$) and double-spin PRP (OR: 0.81 [0.10, 6.84]; $p = 0.85$). In terms of pseudarthrosis, the risk was increased significantly by PRP in both instrumented PLF (OR: 2.03 [1.09, 3.76]; $p = 0.03$) and non-instrumented PLF (OR: 9.70 [1.64, 57.39]; $p = 0.01$). However, subgroup analysis showed no difference ($z = -1.63$). Likewise, there was no difference between single-spin (OR: 1.68 [0.78, 3.62]; $p = 0.19$) and double-spin PRP (OR: 4.58 [0.51, 41.30]; $p = 0.17$). PRP did not reduce the surgery revision rates (OR: 1.47 [0.70, 3.10]; $p = 0.31$) or graft resorption rates (OR: 1.54 [0.24, 9.75]; $p = 0.65$).

Conclusion: PRP does not significantly improve fusion success in PLF, regardless of instrumentation or preparation methods. However, PRP is associated with an

increased pseudarthrosis risk, with no difference between instrumentation types or preparation methods. Additionally, PRP does not significantly affect the risk of surgery revision or graft resorption. Future high-quality RCTs should be conducted to address variability, suggesting cautious use of PRP in PLF until further evidence emerges.



Po3_1.38

Optimizing Cage Material and Surgical Approach (Open vs. MIS) in TLIF: A Network Meta-Analysis of PEEK, Titanium, TiPEEK, and 3D-Printed Titanium

Chawakrit Pansritoom

Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

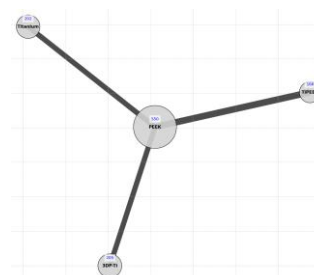
Abstract

Background: TLIF is widely used for degenerative lumbar spine disorders. The choice of cage materials significantly affects fusion success and complication rates. However, evidence remains inconclusive regarding the superiority of one material over another.

Objectives: To compare the efficacy and safety of four cage materials—**PEEK, Titanium, TiPEEK, and 3D-Printed Titanium**— and to evaluate the impact of **Open vs. MIS TLIF** on cage performance through a pre-specified subgroup analysis.

Methods: Following PRISMA-NMA guidelines, a systematic search was conducted across PubMed, MEDLINE, and Scopus until January 2025. Using a frequentist framework with STATA16, four cage types were compared across Open and MIS TLIF approaches. The outcomes included **fusion success at final follow-up (≥ 12 months), ODI improvement, VAS back and leg pain improvement, revision surgery, and complication rates** (i.e., graft subsidence, pseudarthrosis, infection, and dural tears).

Results: 13 studies (5 RCTs, 1 prospective cohort, and 7 retrospective cohorts) involving 1,086 patients were included. **3D-Printed Titanium** showed the best outcomes, with the highest fusion success (OR = 4.21, 95% CI: 2.35–7.52, $p < 0.001$), ODI improvement (OR = 3.45, 95% CI: 1.95–6.12, $p < 0.001$), and reductions in VAS back pain (OR = 3.78, 95% CI: 2.01–6.90, $p < 0.001$) and leg pain (OR = 3.52, 95% CI: 1.92–6.45, $p < 0.001$), using **PEEK as the reference material**. **TiPEEK** ranked second, significantly improving fusion success (OR = 3.18, 95% CI: 1.95–5.24, $p < 0.001$), ODI improvement (OR = 2.89, 95% CI: 1.67–5.12, $p = 0.001$), and VAS reductions. Lastly, **Titanium** ranked third across all outcomes. Revision surgery and complication rates were lowest with 3D-Printed Titanium (OR = 0.24, 95% CI: 0.11–0.53, $p < 0.001$; OR = 0.31, 95% CI: 0.17–0.58, $p < 0.001$), followed by TiPEEK and Titanium respectively. Subgroup analysis revealed significant improvements favoring **MIS TLIF over Open TLIF regardless of cage material**, with higher fusion success (OR = 1.45, 95% CI: 1.08–1.95, $p = 0.04$) and ODI improvement (OR = 1.38, 95% CI: 1.01–1.88, $p = 0.05$). While trends in VAS scores, revision rates, and complication rates favored MIS TLIF, these were not statistically significant. Based on SUCRA rankings, **3D-Printed Titanium** consistently ranked



highest across outcomes, followed by **TiPEEK**, **Titanium**, and **PEEK**.

Conclusion: **3D-Printed Titanium** led across all outcomes, followed by **TiPEEK**, **Titanium**, and **PEEK**. **MIS TLIF** outperformed **Open TLIF**, with 3D-Printed Titanium and MIS TLIF providing optimal results. *Network plot illustrating the direct comparisons between cage materials*

Po3_1.39

Long-Term Outcomes of Cortical Bone Trajectory versus Traditional Pedicle Screws in Lumbar Fusion: A Systematic Review and Meta-Analysis

Chawakrit Pansritoom

Chulalongkorn University, Bangkok, Thailand

Abstract

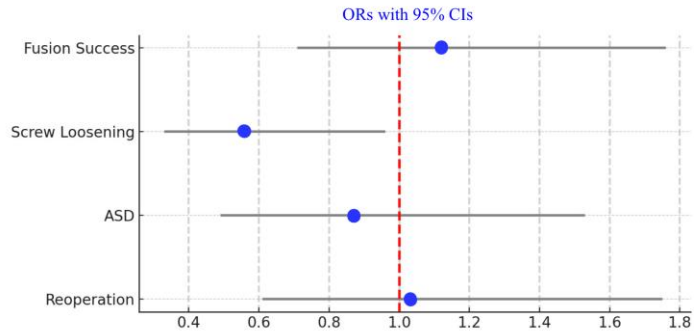
Introduction: Lumbar fusion is a common treatment for degenerative lumbar spine disorders. Cortical bone trajectory (CBT) offers potential advantages over traditional pedicle screws (TPS), including reduced muscle dissection and improved screw purchase, especially in osteoporotic bone. However, long-term comparative outcomes of CBT versus TPS remain unclear.

Objectives: This meta-analysis compares the long-term efficacy and safety of CBT versus TPS in lumbar fusion, focusing on fusion success, hardware complications, adjacent segment disease (ASD), and patient-reported outcomes at ≥ 24 months follow-up.

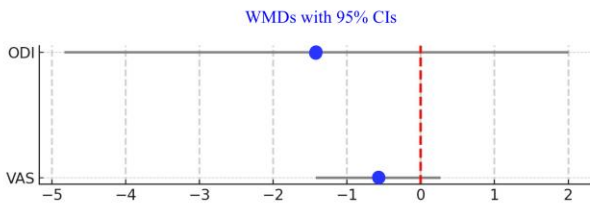
Methods: Following PRISMA guidelines, PubMed, MEDLINE, and Scopus were searched through February 2025. Studies comparing CBT and TPS with ≥ 24 months follow-up were included. The primary outcome was fusion success; secondary outcomes included screw loosening, ASD, reoperation rates, and patient-reported outcomes (ODI, VAS). Data were pooled using random-effects models, with odds ratios (OR) and weighted mean differences (WMD); heterogeneity was assessed with I^2 statistics.

Results: Eight studies (3 RCTs, 5 cohort studies; 652 patients) were included. Fusion success was similar between CBT and TPS (OR: 1.12 [0.71, 1.76]; $p = 0.63$). CBT had lower screw loosening rates (OR: 0.56 [0.33, 0.96]; $p = 0.04$), while ASD (OR: 0.87 [0.49, 1.53]; $p = 0.62$) and reoperation rates (OR: 1.03 [0.61, 1.75]; $p = 0.91$) showed no differences. ODI (WMD: -1.42 [-4.83, 2.00]; $p = 0.42$) and VAS (WMD: -0.58 [-1.42, 0.27]; $p = 0.18$) scores were comparable. Heterogeneity ranged from low to moderate ($I^2 = 0-45\%$).

Conclusions: CBT and TPS yield comparable long-term fusion success and patient-reported outcomes. CBT reduces screw loosening risk without affecting ASD or reoperation rates, making it a viable alternative to TPS, especially for patients at higher hardware failure risk. Further long-term RCTs are needed to confirm these findings.



Forest plot comparing Cortical Bone Trajectory (CBT) vs. Traditional Pedicle Screws (TPS) in lumbar fusion for long-term outcomes, including fusion success, screw loosening, adjacent segment disease (ASD), and reoperation rates.



Forest plot comparing Cortical Bone Trajectory (CBT) vs. Traditional Pedicle Screws (TPS) in lumbar fusion for patient-reported outcomes, including improvements in Oswestry Disability Index (ODI) and Visual Analog Scale (VAS) scores.

Po3_1.40

The Importance of Psoas Muscle on Low Back Pain: a Single-Center Study on Lumbar Spine MRI

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Abstract

INTRODUCTION: Low back pain (LBP) prompts numerous magnetic resonance imaging (MRI) examinations of the lumbosacral spine as it is an highly prevalent symptom. The specific role of soft tissues, particularly muscles, in LBP remains unclear, and detailed exploration of each MRI-derived parameter's contribution to LBP intensity is lacking. This study seeks to examine the relationship between soft tissue status, as derived from MRI parameters, and LBP. The focus is on evaluating the individual impact of parameters such as the area of paravertebral and psoas muscles, extent of intramuscular fat infiltration, and disc degeneration from L1 to S1 on LBP.

METHODS: This retrospective observational study, conducted at a University Hospital, involved 94 patients experiencing LBP due to degenerative disc disease who underwent MRI scans of the lumbosacral spine. Imaging was performed with a 1.5 Tesla scanner. Patients provided symptom information through a questionnaire and assessed their pain intensity using Visual Analogue Scale (VAS). VAS scores were classified as mild, moderate, or severe, with cutoff values of 3.8 and 5.7 based on literature. Biometric data, encompassing weight and height, were recorded for calculating the body mass index (BMI). The ratios between intramuscular fat infiltration and net muscle area were computed.

RESULTS: The stepwise analysis revealed that increasing psoas net area was associated with lower VAS levels [odds ratio (OR): 0.94; 95% confidence interval (CI): 0.90-0.98; p=0.005], and an increase of one square centimeter of total psoas area resulted in a greater probability of reporting a mild (+1.21%; 95% CI: 0.37, 2.05%) or a moderate VAS (+0.40%; 95 % CI: -0.02, 0.82%), Furthermore, a more severe VAS was associated with a higher BMI (OR: 1.13; 95% CI: 1.00-1.27).

DISCUSSION: Our research reveals a connection between LBP and the status of paravertebral and psoas muscles as indicated by MRI parameters. The significance of the psoas muscle in spine stabilization is underscored, and its correlation with clinical symptoms in LBP patients is evident. These insights have the potential to inform future studies and enhance treatment strategies for individuals with LBP, with the prospect of mitigating the effects on disability, quality of life, and socio-economic burden.

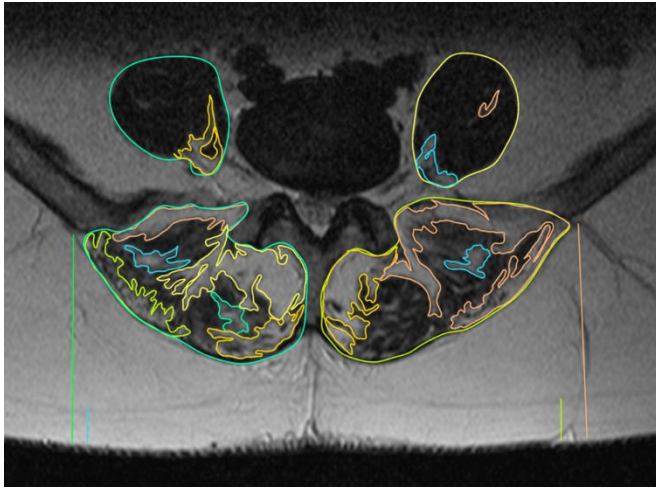


Figure 1: Cross-sectional total muscle area of paravertebral and psoas muscles, intramuscular adipose infiltration and the maximum thickness of posterior subcutaneous adipose tissue.

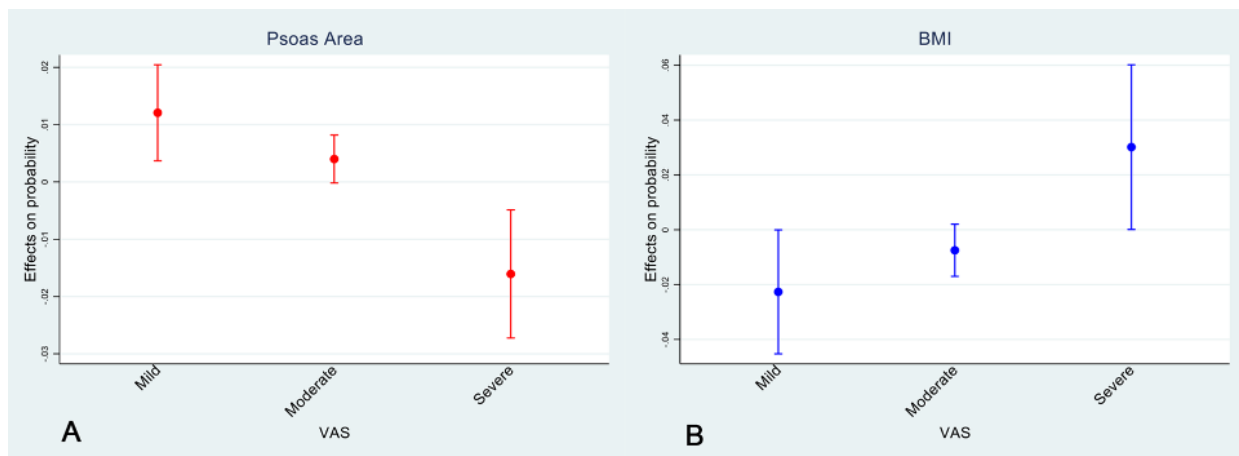


Figure 2: Marginal effects of total psoas area (one cm² increase) (A) and BMI (one point increase) (B) on the probability of mild, moderate, and severe VAS score.

Po3_1.41

The Fear-Avoidance Model in Patients with Low Back Pain: A Systematic Literature Review and Meta-Analysis

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Abstract

Background Low back pain (LBP) is a leading cause of disability worldwide. The Fear-Avoidance Model (FAM) is key to understanding the psychosocial aspects of chronic pain. According to FAM, individuals who excessively fear pain may avoid physical activities, leading to deconditioning and prolonged pain. This systematic review and meta-analysis evaluate the impact of FAM on clinical outcomes, such as pain, disability, and quality of life, in LBP patients.

Methods A systematic review was conducted across PubMed/MEDLINE, Scopus, Web of Science, and Cochrane databases, with no time restrictions, up to March 2024. Studies exploring the correlation between FAM factors (fear-avoidance, kinesiophobia, pain catastrophizing) and clinical outcomes (pain and disability) were included. Study selection was managed using CADIMA, and qualitative assessments were performed using Joanna Briggs Institute tools. Meta-analysis was conducted in RStudio.

Results Our review included 49 studies, of which 16 were analyzed in the meta-analysis. FABQa showed a strong correlation with ODI ($r = 0.42$, $p < 0.05$) and a moderate correlation with VAS ($r = 0.28$, $p < 0.05$). FABQw had a moderate correlation with ODI ($r = 0.44$, $p < 0.05$) and a positive correlation with VAS ($r = 0.28$, $p < 0.05$). TSK showed a lower correlation with ODI ($r = 0.24$, $p < 0.05$) and a moderate correlation with VAS ($r = 0.38$, $p < 0.05$). PCS exhibited a strong correlation with VAS ($r = 0.51$, $p < 0.05$).

Conclusions This review confirms that fear-avoidance beliefs significantly impact pain and disability. The strong correlation between PCS and VAS underscores the role of pain catastrophizing in intensifying perceived pain. These findings highlight the need to address psychosocial factors like fear-avoidance beliefs and pain catastrophizing in managing LBP. For healthcare professionals, this emphasizes the importance of holistic care, integrating both physical rehabilitation and psychological support to enhance recovery outcomes.

Study funded by PNRR-MAD-2022-12376692_VADALA' – CUP F83C22002470001 project

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Po3_1.43

Advancements in AI for Low Back Pain Integrating Automated Muscle Analysis and Disc Identification.

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Abstract

Introduction: Low back pain (LBP) is an endemic musculoskeletal condition and a global leading cause of disability. To enhance early diagnostic and treatment capabilities, artificial intelligence (AI) has emerged as a transformative tool. In this study, we developed a fully automated algorithm for disc identification and lumbar paraspinal muscle (PSM) segmentation from lumbar magnetic resonance imaging (MRI) scans.

Methods: After Gaussian filtering for noise reduction, a 2D isotropic filter is applied. Column-wise average intensity is calculated, allowing to determine reference coordinates for the spinal canal, which guide a composite function for curve identification. A linear approximation is made for the upper 20% of image rows, followed by a 3rd-degree polynomial for the remaining curve. The resulting shape is used to create a mask, shifting it leftwards to select the region encompassing the vertebrae and discs based on intensity. Similarly, total intensity per pixel row is calculated, identifying intensity peaks (vertebrae) and valleys (discs). Local minimal intensity reveals the vertical coordinates of intervertebral discs.

The focus then shifts to L4-L5 level. A U-Net architecture is employed for the automated segmentation of lumbar PSM. This algorithm processes MRI data, distinguishing between muscle and non-muscle structures. The outcome is a precise delineation of muscle boundaries, forming the foundation for subsequent analyses.

The second segmentation task involves distinguishing between muscle and fat subregions within regions of interest (ROIs), based on a visible contrast difference. Otsu's thresholding method is applied to PSM ROI. The raw fat mask is then propagated using a recursive empirical region-growing algorithm. This process applies iteratively until no substantial area increase is detected. Hyperparameters are optimized through grid search, enhancing accuracy metrics for the final setup. T2-weighted lumbar MRI scans of 91 subjects were considered for the preliminary investigation, and images of 100 patients from a public dataset were utilized for further validation.

Results: We achieved accurate automated disc identification (100%) and subsequent selection of axial slices. At L4-L5 level, the U-Net-based muscle segmentation achieved an

average DICE score >95% on the internal dataset, and >94% on the public dataset. Adipose infiltration assessments complemented these findings, contributing to a comprehensive understanding of the musculoskeletal dynamics.

Conclusions: This detailed methodology integrates cutting-edge AI techniques. The automated disc-to-axial slice association streamlines the process, allowing for targeted analysis at L4-L5 level. The implications for personalized treatment plans and the broader landscape of AI-enabled healthcare interventions will shed new lights potentially revolutionizing LBP management.

Po3_1.44

Cross-Cultural Adaptation and Validation of the Spanish Version of The Early-Onset Scoliosis Self Report Questionnaire (EOSQ-SELF)

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Abstract

PURPOSE: The Early-Onset Scoliosis Self-Report Questionnaire (EOSQ-SELF) is a self-assessment tool for measuring health-related quality of life (HRQoL) in children with early-onset scoliosis (EOS). It complements the 24-item Early-Onset Scoliosis Questionnaire (EOSQ-24), which relies on proxy reporting. Originally validated in English and Chinese, this study aims to translate, culturally adapt, and validate the EOSQ-SELF for Spanish-speaking Hispanic populations, enhancing its applicability in assessing HRQoL in children with EOS.

METHODS: A cross-sectional study was conducted from August to September 2024 with children aged 7 to 14 years diagnosed with EOS, recruited from pediatric orthopedic centers in Puerto Rico. The EOSQ-SELF was translated and culturally adapted using forward-backward translation and expert review. Participants completed the questionnaire twice, with a two-week interval. Reliability was evaluated through internal consistency (Cronbach's α) and test-retest reliability. Content validity was assessed by an expert panel, while discriminative ability was analyzed using nonparametric tests and regression. (Approved by IRB)

RESULTS: 40 participants (mean age 11 years, 53.3% female) provided valid responses. The mean Cobb angle was $60.12^\circ \pm 18.30^\circ$. The Spanish EOSQ-SELF demonstrated excellent internal consistency (Cronbach's $\alpha = 0.937$) and effectively distinguished between different scoliosis severities and types.

CONCLUSIONS: The Spanish EOSQ-SELF is a reliable and valid tool for assessing self-reported HRQoL in Hispanic children with EOS. It has signific.

Po3_1.45

Thoracic pedicle screw anchorage using the new modified slide technique

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Abstract

BACKGROUND. Pedicle screws have earned high acceptance in a broad variety of spinal surgeries. Nevertheless, placement in the thoracic spine remains a challenge due to the difficult anatomical conditions, particularly if deformities are present. Several freehand insertion techniques have been described to identify the pedicle entry point and to minimize the risk of screw misplacement. However, some techniques might compromise pedicle screw anchorage, while allowing safe screw placement. This study investigated pedicle screw anchorage of screws placed with the new "Modified Slide"-technique (MS) and compared it to the Slide-technique by Vialle et al., the Funnel-technique as proposed by Gaines et al. as well as the conventional technique.

MATERIAL AND METHODS. Forty-five fresh frozen human thoracic vertebrae (Th4–Th11; mean age: 69.4 years $\pm$ 14; mean BMD: 99.97 mg/ccm $\pm$ 22.96) were used in this study. For pairwise comparisons, one randomly selected pedicle of each vertebra was instrumented using the new MS-technique. The contralateral side was instrumented using either the Funnel-technique, the Slide-technique or the conventional technique resulting in three groups of 15 vertebrae, respectively. Each screw was tested using cyclic cranio-caudal loading with an initial load ranging from -50 N to +50 N with stepwise increasing compressive load (5 N every 100 cycles) until screw loosening was detected. A Wilcoxon signed-rank test was then performed to compare anchorage.

RESULTS. Screws implanted using the MS-technique sustained significantly higher loads than those using the Slide-technique (460.3 N $\pm$ 160.4 vs. 253.0 N $\pm$ 95.2; $p < 0.001$) and the Funnel-technique (440.0 N $\pm$ 157.6 vs. 261.0 N $\pm$ 105.2; $p < 0.001$) but lower loads than screws conventionally implanted (442.3 N $\pm$ 94.0 vs. 538.3 N $\pm$ 117.6; $p = 0.008$).

DISCUSSION. In terms of screw anchoring, the MS-technique is superior to the Funnel-technique and the Slide-technique. Even though screws implanted using the new "Modified Slide"-technique sustained significantly lower loads than conventionally implanted screws they can sustain higher loads than those measured in daily activities while still allowing safe screw implantation. Therefore the "Modified Slide"-technique could be a helpful assistance technique for safe pedicle screw placement.

Po3_1.50

Accuracy of subaxial cervical pedicle screw placement with computer navigation system

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Abstract

Posterior cervical instrumented fixation of cervical spine using subaxial CPS was performed in 59 pts at our institute. The mean age at surgery was 64 years. The clinical diagnosis was CSM with instability or destructive spondyloarthropathy in 16 pts, CSM associated with cerebral palsy in 8 pts, rheumatoid arthritis (RA) in 8 pts, cervical deformity 3 pts, trauma in 11 pts, neoplastic destruction in 13 pts. Degeneration group (35 pts) is CSM, RA and deformity patients. Non-degeneration group (24 pts) is trauma and neoplastic patients. CPSs were placed using a preoperative 3D CT-based navigation system (preoperative group) during the first study period and using an intraoperative 3D CT-based navigation system (O-arm group) during the second study period.

Result. The radiological accuracy of CPS placement was evaluated using postoperative CT scans and was defined retrospectively by a grading scale originally proposed by Neo et al. A total of 164 CPSs were placed in preoperative group, and a total of 132 CPSs in O-arm group, so that 296 CPSs were placed in the spine level of C3 to C7. In preoperative group, accuracy of CPS placement was 91.5 % (150 of the 164 screws) in grade 0, 5.4 % (9 of the 164 screws) in grade 1, 1.2 % (2 of the 164 screws) in grade 2, and 1.8 % (3 of the 164 screws) in grade 3. In O-arm group, accuracy of CPS placement was 97.0 % (128 of the 132 screws) in grade 0, 2.2 % (3 of the 132 screws) in grade 1 and 0.8 % (1 of the 132 screws) in grade 3. Malposition of CPS in degeneration group was identified in 16 pts of the 35 pts. Malposition of CPS in non-degeneration group was identified in 0 pts of the 24 pts. There is no difference in accuracy in navigation. Accuracy was different in disease.

Po3_1.51

Mechanosensitive ion channel PIEZO1 regulates ossification of Annulus Fibrosus

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Abstract

Background: Intervertebral discs are subjected to daily mechanical stress, which can lead to degeneration and calcification of the annulus fibrosus. While the roles of mechanosensitive ion channel PIEZO1 has been reported in various organs, its involvement in the annulus fibrosus remains unclear. This study aimed to elucidate the role of PIEZO1 in the annulus fibrosus.

Methods: Annulus fibrosus cells were harvested from 10-week-old Wistar rats and only passage 0 cells were used for *in vitro* experiments to investigate the functional activity of PIEZO1. The effects of cyclic tensile stretch (CTS) were evaluated using RT-qPCR. To confirm that these effects are mediated by PIEZO1, RNA interference (siPIEZO1) was combined with CTS. Additionally, the influence of PIEZO1 activation by agonist (Yoda1) was analyzed through RNA sequencing, RT-qPCR and Western blotting. The impact of PIEZO1 activation on BMP2-induced ossification was also assessed using RT-qPCR, Alizarin Red staining and immunocytochemistry.

Results: Moderate tensile stretch (2%, 1 Hz, 12 h) suppressed the expression of *Runx2* and *Osx*, key ossification markers. This CTS-induced suppression was partially reversed by siPIEZO1, confirming the involvement of PIEZO1. Gene Set Enrichment Analysis of RNA sequencing data identified that differentially expressed genes by the PIEZO1 activation were associated with negative regulation of ossification. RT-qPCR and Western blotting further validated the downregulation of *Runx2*. In BMP2-induced ossification experiments, Yoda1 suppressed the upregulation of *Osx* and *Alp* expression, as well as reduced Alizarin Red staining. Immunocytochemistry revealed that Yoda1 inhibited BMP2-induced nuclear translocation of pSMAD1/5/9, and this effect was attenuated by the calcineurin inhibitor cyclosporine.

Conclusion: Mechano-transduction via PIEZO1-mediated intracellular Calcium influx activates calcineurin, leading to dephosphorylation of pSMAD1/5/9. This suppresses the BMP signaling pathway and regulates ossification. These findings suggest a pivotal role for PIEZO1 in the control of ossification of the annulus fibrosus.

Po3_1.52

Targeting cellular senescence to combat osteoarthritis and intervertebral disc degeneration

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Abstract

Introduction. Osteoarthritis and low back pain, of which intervertebral disc (IVD) degeneration is strongly associated, are leading causes of disability worldwide, leading to substantial pain, decreased mobility and major impacts on individuals' quality of life. However, despite the debilitating effects, current treatments are inadequate and do not target the underlying biological causes. Osteoarthritis and IVD degeneration share several commonalities and are characterised by cellular dysfunction that leads to tissue destruction, mechanical damage, and pain. We hypothesize that by targeting a common pathway in the development of osteoarthritis and IVD degeneration known as cellular senescence, where cells no longer divide but continue to produce catabolic factors that drive degeneration, we will be able to halt the progression of both diseases. One such pathway is cellular senescence where growth and division is halted, yet cells still produce vast amounts of catabolic factors, known as senescence associated secretory phenotype (SASP). Within both osteoarthritis and IVD degeneration senescence and the production of SASP is known to contribute to and exacerbate disease progression. We aim to test a range of anti-senescence drugs (senolytics) and novel compounds to target and remove the dysfunctional cells within human knee and IVD tissue explant culture models.

Methods. An osteoarthritis model was developed by co-culturing osteochondral explants with synovium taken from knee replacement surgery. Co-cultures, and IVD tissue explants, were treated for 1w with previously known senolytic drugs and novel compounds to specifically target the cGAS-STING pathway. Cell viability, gene and protein expression, and secretome analysis were utilised to determine the effects of senolytics on tissues.

Results. Osteochondral and synovial co-cultures were viable and maintained their phenotype for at least 3w in culture. Cell viability was not adversely affected by any senolytic treatments. Following treatment, the gene and protein expression of specific senescence and catabolic markers were altered following treatment compared to untreated controls.

Discussion. We have identified that a specific pathway linked to both osteoarthritis and IVD degeneration, cellular senescence, can be targeted using senolytic compounds which have shown potentially disease-modifying effects following treatment. Our development of a human osteoarthritis co-culture model will also enable the rapid testing of other novel therapies within a physiologically relevant *ex vivo* system.

Po3_1.55

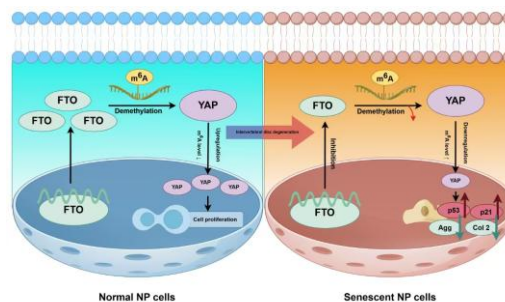
Mechanism of FTO-Mediated m6A Demethylation Regulation of YAP in Nucleus Pulposus Cell Senescence

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Abstract

Objective: To investigate the changes in m⁶A methylation levels, as well as the expression patterns of FTO and YAP during the senescence of nucleus pulposus cells; to explore the regulatory role of FTO and YAP expression changes in nucleus pulposus cell senescence; and to examine the dose-response relationship between FTO-mediated m⁶A demethylation and YAP expression levels.



m6A-mediated regulation of nucleus pulposus cell senescence

Methods: Human nucleus pulposus tissue samples with varying degeneration degrees and rat tail intervertebral disc nucleus pulposus tissue samples were collected. The m6A methylation level in rat samples was assessed using the Dot Blot method. Eight-week-old SD rat nucleus pulposus cells were isolated, cultured, and subjected to IL-1 β intervention to establish an inflammation-induced premature senescence model. Histological analyses, including HE and SO staining along with immunohistochemistry, were conducted to evaluate the senescence levels of nucleus pulposus tissue and the expression of m6A methyltransferases. Key genes associated with cell senescence were identified through m6A methylation sequencing. Small interfering RNA was employed to downregulate FTO expression, while lentivirus was utilized for YAP overexpression. Changes in senescence-related phenotypes were assessed using SA- β -gal staining, with Western Blot, ELISA, and immunofluorescence techniques employed to detect the alterations in FTO, YAP, senescence-related markers (p53, p16), and the secretion capacity of extracellular matrix components (Collagen II, Aggrecan).

Results: m⁶A methylation levels significantly increased in the aged rat nucleus pulposus tissue. Both FTO and YAP expression were downregulated in degenerated nucleus pulposus tissue. In nucleus pulposus cells, compared to the control group transfected with empty plasmid, both FTO and YAP expressions were significantly reduced in the FTO-specific siRNA transfection group, with notable upregulation of senescence-related phenotypes such as p53 and p16, while the expression of extracellular matrix components Collagen II and Aggrecan was significantly decreased. In the FTO-specific siRNA transfection group, overexpression of YAP resulted in a significant reduction in senescence-related phenotypes

(p53, p16) compared to the FTO knockdown group, while the expression of extracellular matrix components (Collagen II, Aggrecan) was significantly increased.

Conclusion: The m⁶A levels are significantly elevated in the aged rat nucleus pulposus tissue. In rat nucleus pulposus cells, after IL-1 β induced senescence, the expression of FTO and YAP was markedly reduced, while the expression of corresponding senescence phenotype proteins increased significantly, leading to a decline in the synthetic capacity of the extracellular matrix. FTO may alleviate YAP methylation levels, mitigating nucleus pulposus cell senescence and reducing intervertebral disc degeneration.

Po3_1.57

Fusion Rate of Biphasic Calcium Phosphate Bone Graft with Needle-Shaped Submicron Topography in Interbody Lumbar Fusion for Degenerative Disc Disease: A Single-Center Retrospective Review

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Abstract

Objective: Iliac crest bone graft (ICBG) is considered the gold standard graft for spinal fusion. However, limitations in the availability of ICBG and morbidity associated with the procedure have led to the development of alternative bone graft options. The purpose of this study is to determine the 12-month fusion rate and clinical outcomes following lumbar interbody fusion utilizing synthetic biphasic calcium phosphate graft with submicron needle-shaped surface topography (BCPμm).

Methods: A single-center retrospective review was conducted to identify patients who underwent lumbar interbody arthrodesis for degenerative disc disease with BCPμm with or without the addition of autograft. Post-operative x-rays were obtained at 3, 6, and 12 months, and computed tomography (CT) scans were obtained at 12 months post-operative. Fusion was determined by an independent neuroradiologist using the CT alpha-numeric classification based on the presence of bridging bone. Secondary outcomes included changes in Visual Analogue Scale (VAS), length of stay, and fusion of all treated levels.

Results: Sixty patients with 105 treated levels were included in the analysis (57% female, 43% male). The average age of patients was 63, and average BMI was 30.1. Patients had an average of three comorbidities and two levels fused. Fifty seven percent of the cases were anterior lumbar interbody fusions (ALIFs), 26% were transforaminal lumbar interbody fusion (TLIF), and 17% were lateral lumbar interbody fusion (LLIF). Bony ingrowth or bridging trabecular bony fusion without evidence of supplemental fixation failure was demonstrated in 95.2% of levels treated (100/105). Fusion rates were not statistically different between procedure types ($p=0.95$). Five patients reported complications, and VAS improved by a mean of 24.18 ($p<0.0001$) six months post-operative.

Conclusions: BCPμm may offer an alternative to ICBG in patients undergoing spinal interbody fusion. This study demonstrates a fusion rate of 95.2% with a low overall complication rate in a diverse population including patients with multiple comorbidities.

Po3_1.58

Value of different preoperative bone evaluation methods in predicting intraoperative screw insertion torque-a prospective clinical comparative trial

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Abstract

Background Context. Establishing good screw-bone structural stability is conducive to reducing the risk of postoperative screw loosening. Screw insertion torque is an objective index for evaluating screw-bone structural stability. Therefore, accurate prediction of screw insertion torque can improve the preoperative evaluation of patients, optimize the surgical plan, and improve the surgical effect. At present, the correlation between different bone assessment methods and screw insertion torque is unclear.

Purpose. The aim of this study was to evaluate the correlation between different bone assessment methods and screw insertion torque and to optimize the predictive performance of screw insertion torque through mathematical modeling combined with different radiology methods.

Design. Prospective cross-sectional study

Patient samples. Seventy-seven patients with preoperatively available DXA, CT and MRI data who underwent spinal fixation surgeries between October 2022 and September 2023 and 357 sets of screw data were included in this analysis.

Outcome Measures. Spinal, vertebrae-specific and screw trajectory's BMD were measured preoperatively by different imaging modalities. Intraoperative screw insertion torque was measured using an electronic torque wrench.

Methods. Pearson linear correlation, scatter plots and univariate linear regression were used to evaluate the correlation between different bone evaluation methods and screw insertion torque. Different bone evaluation methods were fitted into the prediction model of screw torque and the related equations were obtained.

Results. Screw insertion torque had the strongest positive correlation with the volumetric bone mineral density (vBMD) of the screw trajectory (Pedicule screw insertion torque (PSIT): $R = 0.618$, $p < 0.001$; Terminal screw insertion torque (TSIT): $R = 0.735$, $p < 0.001$). A weak negative correlation was found between the screw insertion torque and level specific vertebral bone quality (VBQ) (PSIT: $R = -0.178$, $p = 0.001$; TSIT: $R = -0.147$, $p = 0.006$). We also found that the PSIT was strongly correlated with the TSIT ($R = 0.812$, $p < 0.001$).

Conclusions. Compared to other bone quality assessment methods, the strongest correlation was found between the screw trajectory vBMD and screw insertion torque. We further optimized preoperative assessments by constructing a mathematical model to better predict screw insertion torque.

Po3_1.59

Macrophage-derived Legumain Attenuates Intervertebral Disc Degeneration by Regulating Stress-induced Catabolism and Metabolism Imbalances of Nucleus Pulposus cells via the Integrin $\alpha\beta3$ /RhoA/ROCK1 Pathway

Peiyang Wang, Zhiyang Xie, Lei Liu, Cong Zhang, Yuntao Wang, Xiaotao Wu

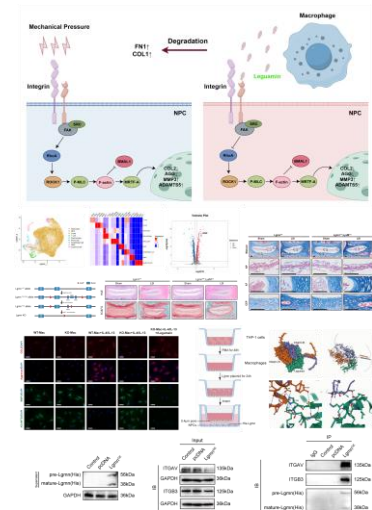
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Abstract

Introduction: Intervertebral disc degeneration (IVDD) is a leading cause of low back pain with significant implications for individuals and society. Macrophages are key players in maintaining tissue homeostasis by sensing and regulating variables such as mechanical stress and osmotic pressure. This study aims to explore the role and mechanism of macrophage-secreted Legumain (LGMN) in IVDD.

Methods: Single-cell transcriptome analysis was utilized to identify differentially expressed genes in macrophages during IVDD. Conditional knockout of LGMN in macrophages was achieved using LysM-Cre and LGMN-Flox transgenic mice. Lumbar spine instability (LSI) surgery was conducted on LGMN cko mice, with samples from LSI segments collected after 12 weeks for histological and immunohistochemical analysis to assess IVDD progression. In vitro, a cellular compression model was employed to simulate mechanical stress on nucleus pulposus cells. Co-culture of macrophages and nucleus pulposus cells from LGMNF/F;LysMCre mice was performed to investigate the role of LGMN. Changes in catabolic and anabolic markers in the nucleus pulposus were assessed using Western blot, RT-PCR, and immunofluorescence. The primary mode of action of LGMN was identified through protein mass spectrometry analysis post-immunoprecipitation of LGMN, further validated by co-immunoprecipitation. The pathway through which LGMN operates was elucidated by transcriptome analysis and confirmed through rescue experiments.

Results: Single-cell transcriptome analysis revealed a significant upregulation of LGMN in macrophages from IVDD patients. Elevated expression of LGMN in macrophages was confirmed in LSI-induced disc degeneration in mice and pressurization-induced rat disc models. Deletion of LGMN specifically in macrophages exacerbated LSI-induced intervertebral disc degeneration, while macrophage-derived LGMN alleviated pressurization-induced catabolic and metabolic imbalances in the nucleus pulposus. Mass spectrometry, immunoprecipitation, and immunofluorescence co-localization studies demonstrated that LGMN primarily interacts with integrin $\alpha\beta3$ of NPCs. Transcriptome and



gene ontology analyses indicated that LGMN inhibits stress-induced activation of the RhoA pathway. In conclusion, macrophage-derived LGMN binds to and inhibits integrin $\alpha\beta 3$ in NPCs, thereby attenuating RhoA-GTP activity under pressurized conditions, inhibiting activation of the RhoA/ROCK1/p-MLC pathway, and maintaining NPC homeostasis.

Conclusions: LGMN may be a novel target for the prevention and treatment of IVDD.

Key Words: Intervertebral disc degeneration; Macrophages; Legumain; RhoA

Po3_1.75

A cadaver feasibility study of extradural contralateral C7 ventral root transfer technique for treating upper extremity paralysis

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Abstract

Study design. Six formalin-fixed cadavers were included in the cadaver feasibility study.

Objective. to ascertain whether extradural contralateral C7 ventral root transfer technique in cervical posterior approach is anatomically feasible.

Summary of background data. Upper limb spastic hemiplegia is a common sequela after stroke. In our previous study, we established a method by transferring contralateral C7 dorsal and ventral roots to affected side C7 dorsal and ventral roots respectively in the posterior cervical approach.

Methods. In the present study, six formalin-fixed cadavers were dissected to confirm the anatomical feasibility. Experimental anastomosis in cadavers was conducted. The pertinent lengths of the extradural nerve roots were measured. The surrounding tissue structures between the extradural CC7 nerve roots and the vertebral artery were observed. Cervical MRI of 60 adults were incorporated into the study to measure the distance between the donor and recipient nerves.

Results. The experimental anastomosis showed that the distance between the donor and recipient nerves is about 1 cm, and a short segment of sural nerve is needed to bridge. The distance between the both sides of the exit of the extradural dura mater is 33.57 ± 1.55 mm. The length of the extradural CC7 ventral root is 22.00 ± 0.98 mm. The ventral distance (vd) and the dorsal distance (dd) of males are 23.98 ± 1.72 mm and 30.85 ± 2.22 mm ($p < 0.05$), while those of females are 23.28 ± 1.51 mm and 30.03 ± 2.16 mm. There are C7 vertebral transverse process and ligaments and other soft tissues between the vertebral artery and the extradural C7 nerve root.

Conclusion. our study showed that the extradural contralateral C7 ventral root transfer technique can theoretically achieve better surgical results under the premise of less trauma, including better recovery of motor function and complete preservation of sensory function.

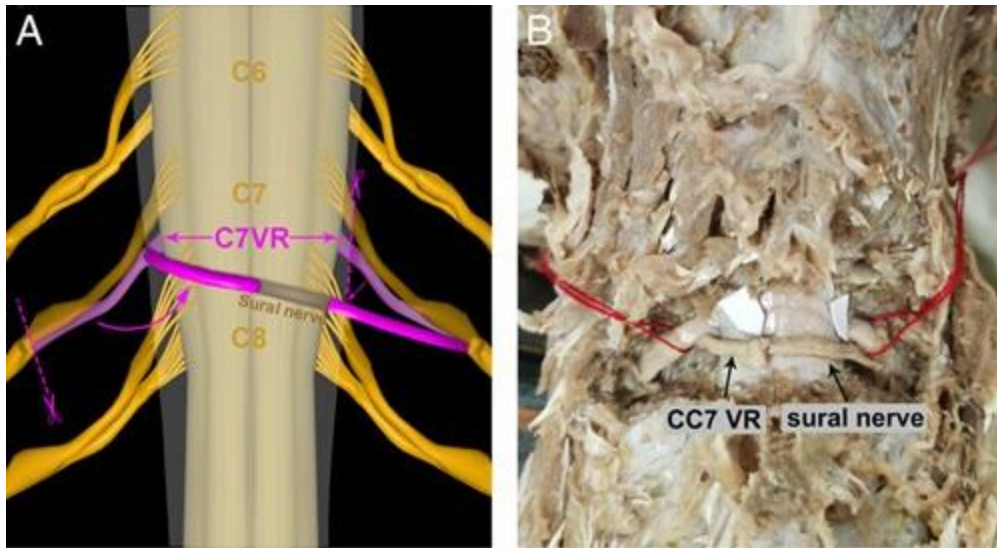


Figure: Schematic and experimental anastomosis between the contralateral and affected sides.

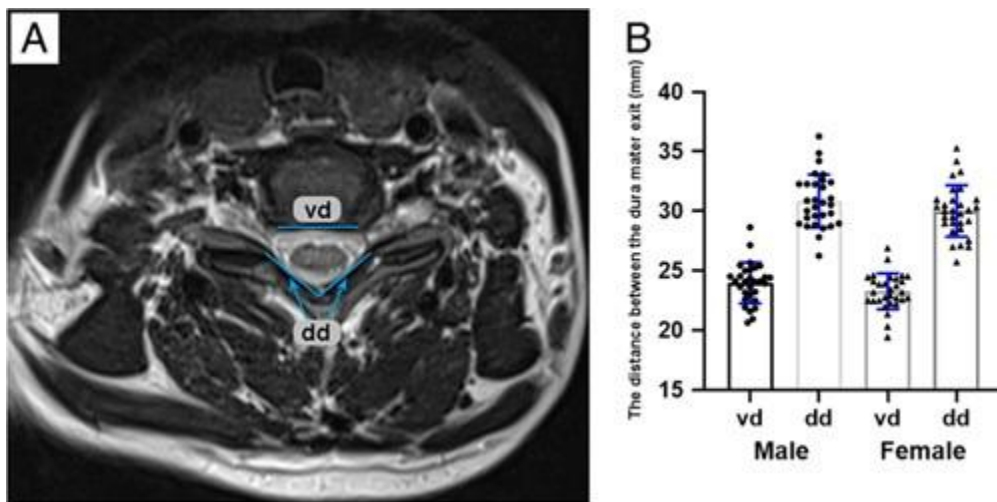


Figure: Measurement of the distances between the two sides using 60 cervical vertebrae magnetic resonance imaging scans.

Po3_1.78

Early Markers of Mechanical Intervertebral Disc Degeneration of Whole Bovine Discs Loaded in a Multiaxial Bioreactor

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Abstract

Introduction: Intervertebral disc (IVD) degeneration (IVDD) is a leading cause of low back pain, with mechanical factors implicated in its development. However, the impact of specific motion patterns on IVDD initiation and progression remains scarcely investigated. We developed a multiaxial bioreactor capable of loading viable bovine IVDs in all 6 degrees-of-freedom of the spine. Repetitive forward bending combined with rotation and axial compression has been predicted to induce a degenerative response in the IVD. Here we investigated the effect of this loading combination on the IVD integrity at different time points, after different numbers of cycles. These data can provide insights into how defined repetitive motion patterns affect IVD health over time, potentially revealing key factors in the timeline of early mechanical IVDD.

Methods: Bovine coccygeal discs were freshly isolated and maintained in sterile culture (N=6). The discs were divided into 4 groups (loaded for 3 consecutive days (S) or free swelling for 3 days (S0); loaded for 7 consecutive days (L) or free swelling for 7 days (L0), Fig 1). Multiaxial loading consisting of repetitive axial compression (0.2 MPa), torsion (0-4°) and flexion (0-6°) was applied at 0.2 Hz to the IVDs in the multiaxial bioreactor (4 hrs/day). Daily IVD height changes, glycosaminoglycan (GAG) and nitric oxide (NO) release in the medium were evaluated. Also, endpoint cell viability (lactate dehydrogenase/ethidium homodimer staining) and gene expression of anabolic, catabolic and inflammatory genes were assessed. Where possible, region-specific changes were assessed.

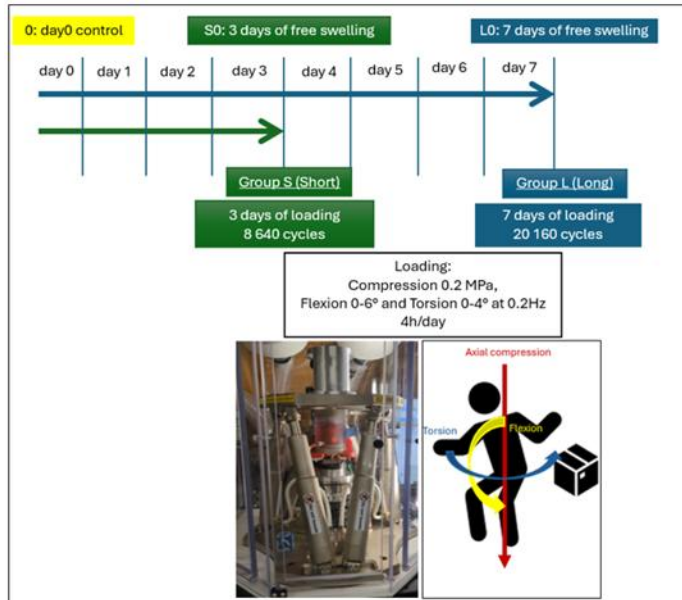


Figure 1: Outline of the experiment. Whole bovine discs are assigned to groups (0: day 0 control. Group S: 3 days of loading. S0: 3 days of free swelling. Group L: 7 days of loading. L0: 7 days of free swelling). Discs are loaded in the Multiaxial Bioreactor for 4h/day.

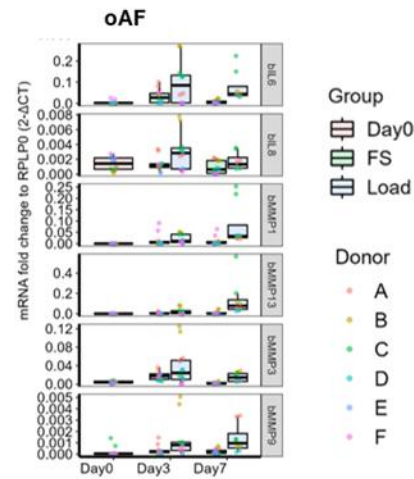


Figure 2: mRNA fold change relative to housekeeping gene in outer annulus fibrosus. N=6. Group Day 0: day 0 Control. Group FS: Free Swelling (S and L). Group Load: Loaded (S and L)

Results: Loaded IVDs displayed an average height loss of $-21.6\% \pm 10.2\%$ during loading. The cumulative release of GAGs and NO in the medium trended to be higher in the loaded discs compared to the unloaded ones. PCR analysis (N=6, Fig. 2) revealed an upregulation of IL-6 and IL-8 gene expression on day 3, and upregulation of catabolic markers (MMP3 on day 3; MMP1, MMP13, MMP9 on day 7) in the outer annulus fibrosus in the loaded group compared to the unloaded group and day 0.

Discussion and Outlook: Multiaxial loading at the selected magnitude induced markers of early IVDD in the outer annulus fibrosus, consistent with prior *in silico* studies (Ristaniemi et al., 2023). Current results suggest an increased inflammation on day 3, followed by catabolic marker upregulation on day 7 in the annulus fibrosus. To confirm this, additional samples will be included, and cell viability will be evaluated in subsequent analyses.

Po3_1.79

Low-magnitude high-frequency vibration and Piezo1 contribute to intervertebral disc degeneration

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Abstract

Introduction: Intervertebral disc (IVD) degeneration is a leading cause of chronic back pain. Low-magnitude high-frequency vibration (LMHFV), commonly used in exercise and rehabilitation, has shown conflicting effects on IVD health – ranging from protective effects¹ to catabolic changes.^{2–4} Taking this, this study aimed to investigate the effect of LMHFV on human IVD cells and tissues, focusing on the mechanosensitive ion channel Piezo1 as a potential mediator.

Methods: Human IVD cells and tissues (n=3–4/group) were treated with LMHFV, Yoda1 (a selective Piezo1 activator), or both. Gene expression was analyzed after 3 days in cell cultures, targeting markers of senescence (*CDKN1A*, *CDKN2A*), inflammation (*PTGS2*, *IL6*, *IL8*), and matrix remodelling (*MMP3*, *MMP13*, *COL1A1*, *COL2A1*). IVD tissues were cultured for 21 days and analyzed for functional and structural changes. Annulus fibrosus (AF) and endplate (EP) stiffness were measured via indentation testing, while AF tensile modulus was assessed by pull-to-failure tests. Collagen and glycosaminoglycan (GAG) content were evaluated by histology and biochemical assays. Statistical analysis: Kruskal-Wallis test.

Results: LMHFV and Yoda1, individually or in combination, significantly increased *CDKN1A* expression compared to controls ($p<0.05$), without altering *CDKN2A*. Yoda1 did not affect inflammatory markers (*PTGS2*, *IL6*, *IL8*) but downregulated *MMP3*, *COL1A1*, and *COL2A1* ($p<0.05$, Fig. 1), and increased AF stiffness ($p<0.05$, Fig. 2A). LMHFV significantly upregulated inflammatory markers ($p<0.05$, Fig. 1A) and showed similar trends for matrix-degrading enzymes and collagen genes (Fig. 1B–C). It also reduced EP stiffness ($p<0.05$, Fig. 2A). Combined LMHFV+Yoda1 treatment decreased EP stiffness compared to Yoda1 alone ($p<0.05$, Fig. 2A). No significant differences were found in AF tensile modulus or in collagen and GAG content across groups (Fig. 2B–D).

Discussion: LMHFV induced catabolic effects in human IVD tissues, including increased inflammation, matrix remodelling, and mechanical weakening. Piezo1 activation contributed to senescence and matrix regulation but did not reproduce the full degenerative effects of LMHFV. These findings align with recent reports identifying Piezo1 as a stress-responsive channel in IVD degeneration^{5,6}, while also suggesting additional

mechanotransduction pathways are involved. Further studies are needed to clarify these mechanisms, relevant for the development of targeted therapies for IVD degeneration.

References: 1. Holguin et al., 2009; 2. McCann et al., 2015; 3. McCann et al., 2017; 4. Widmayer et al., 2023; 5. Wu et al., 2022; 6. Liu et al., 2023.

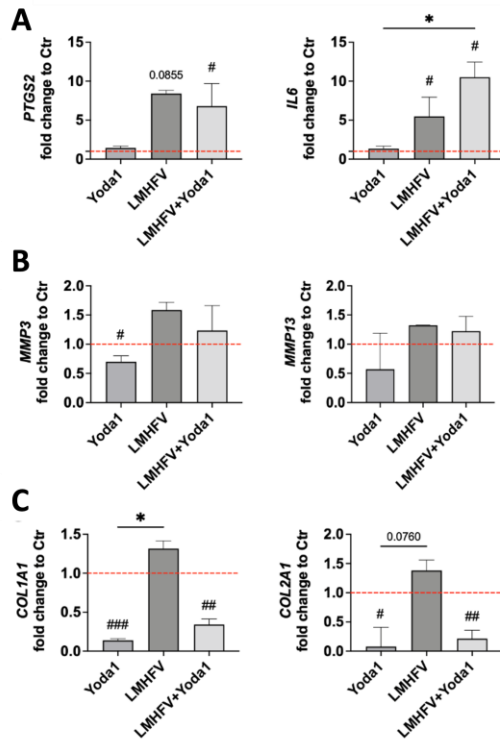


Figure 1: Gene expression of (A) inflammatory markers *PTGS2* and *IL6*, (B) matrix metalloproteinases *MMP3* and *MMP13*, and (C) collagens *COL1A1* and *COL2A1* in human IVD cells. RNA was isolated after 2 days of culture. Levels of messenger RNA (mRNA) were normalized to *GAPDH* and to the control (= 1, dashed line) and are shown as $2^{-\Delta\Delta CT}$. n = 3–4. # p < 0.05, ## p < 0.01, ### p < 0.001 (comparison to control); * p < 0.05 (comparison between treatment groups).

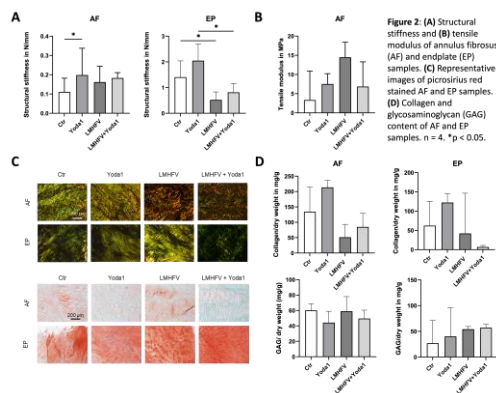


Figure 2: (A) Structural stiffness and (B) tensile modulus of annulus fibrosus (AF) and endplate (EP) samples. (C) Representative images of picrosirius red stained AF and EP samples. (D) Collagen and glycosaminoglycan (GAG) content of AF and EP samples. n = 4, *p < 0.05.

Po3_1.80

Controllable nitric oxide release material relieves intervertebral disc degeneration through antibacterial effect and inhibiting osteoclast activation

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Abstract

INTRODUCTION: Low back pain is prevalent globally and stands as a primary cause of disability. The pathogenesis of intervertebral disc degeneration and Modic changes remains debated, with infection frequently cited as a contributing factor. Prior research has demonstrated that injecting *Cutibacterium acnes* into the intervertebral discs of New Zealand rabbits and rats leads to IVDD and Modic changes. Nitric oxide is critical in numerous physiological and pathological processes and holds promise, particularly for antibacterial purposes. In this study, we developed a controllable nitric oxide donor (PEGNO) capable of local NO delivery under red-light irradiation via photoredox catalysis involving PdTPTBP and CouN(NO)-NO₂ derivatives. This donor was found to halt the progression of IVDD and Modic changes by its antibacterial action and by inhibiting osteoclast differentiation.

METHODS: We covalently linked the NO donor with PdTPTBP in the core of micellar nanoparticles. The hydroxyl groups of CouN(NO)-NO₂ were functionalized with 2-isocyanatoethyl methacrylate to create CouN(NO) and CouN(H) monomers. These monomers were then polymerized using reversible addition-fragmentation chain transfer polymerization, forming copolymers that self-assembled into micelles in aqueous solutions. Post synthesis, the antibacterial efficacy of NO against *C. acnes* was verified, alongside assessments of the donor's biocompatibility and hemolytic activity. In vitro studies confirmed the protective effects of PEGNO under irradiation on disc cells damaged by *C. acnes* and its capacity to inhibit osteoclast differentiation. The effectiveness of PEGNO was further validated in a rat model of IVDD.

RESULTS: Agarose plate assays showed that the NO donor inhibited *C. acnes* proliferation post-irradiation. CCK8 assays and hemolysis tests indicated minimal cytotoxicity at concentrations up to 0.5g/L. In vitro, PEGNO effectively prevented pyroptosis in disc cells co-cultured with *C. acnes*. In the rat IVDD model, PEGNO-treated rats under irradiation showed superior MRI results compared to both the *C. acnes* injection group and the antibiotic-treated group. Histological analyses demonstrated more intact annulus fibrosus, less bone destruction, and healthier nucleus pulposus and cartilage endplates in the PEGNO group.

DISCUSSION: Currently, no ideal treatment exists for chronic intervertebral disc infections, and traditional antibiotics are often ineffective against anaerobic pathogens like *C. acnes*. Nitric oxide, a potential therapeutic gas molecule, is advantageous due to its low toxicity and ease of metabolism. The synthesized PEGNO enabled efficient NO release both in vitro and in vivo, significantly reducing inflammation and cartilage endplate destruction induced by *C. acnes*, thereby delaying the progression of IVDD and Modic changes.

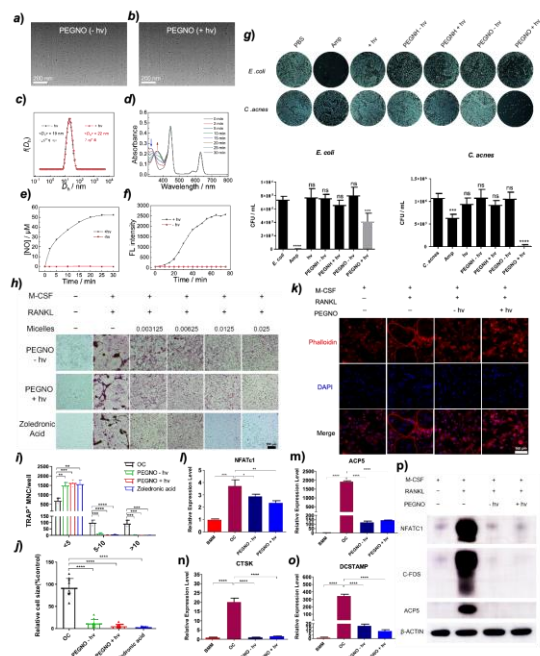


Figure 1. In vitro Antimicrobial and Anti-Osteoclast Activity of PEGNO. TEM images of PEGNO micelles (a) before and (b) after 630 nm irradiation for 30 min. (c) Intensity-average hydrodynamic diameter distributions (DDs) of PEGNO micelles with or without 630 nm irradiation for 30 min. (d) UV-vis spectra, (e) NO release profiles, and (f) fluorescence intensity changes of PEGNO micelles (0.1 g/L) in the presence of sodium ascorbate (10 mM) under 630 nm irradiation. In all cases, the irradiation intensity was 30 mW/cm². (g) Bacterial colony forming unit away of PEGNO against *E. coli* and *C. acnes*. (h) Trap staining to detect osteoclastogenesis of BMDs following the treatment with PEGNO - *hs*, PEGNO + *hs*, and zoledronic acid (0.0125 g/L). (i) Quantification of size of TRAP-positive multinuclear cells ($n = 9$). (j) Quantification of nuclei numbers of TRAP-positive multinuclear cells ($n = 3$). (k) Phalloidin staining of osteoclastogenesis treated with PEGNO with or without 630 nm red light irradiation (30 mW/cm²). Detection of the expression levels of OC differentiation marker genes (l) NFATc1, (m) ACP5, (n) CTSK and (o) DCSTAMP. (p) Western blotting test of NFATc1, C-FOS, ACP5 in osteoclastogenesis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant. All results are representative data generated from at least three independent experiments. Data are presented as mean $\pm$ SD. The one-way ANOVA with the Tukey's multiple comparison test was used for statistical analysis.

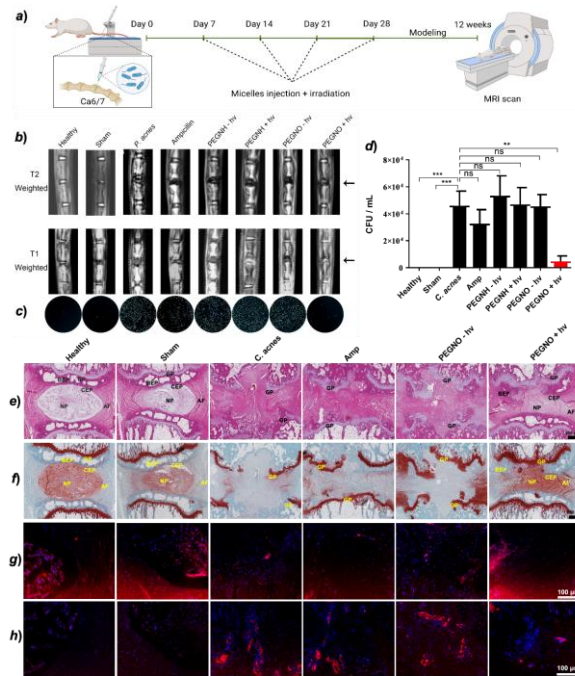


Figure 2. The efficacy of PEGNO in *C. canis*-induced disc degeneration. (a) Experimental timeline of *P. acnes*-induced rat caudal IVD model, created with BioRender.com. (b) Representative T1-weighted and corresponding T2-weighted images of *P. acnes* injection tail disc receiving different treatments at week 12. (c) Photographs of clones on agar plates of *P. acnes* harvested from rat IVD tissue receiving varying treatments. (d) Bacterial colony-forming unit counts of *P. acnes*-infected caudal discs. Data are shown as mean $\pm$ SD. (n = 3); ** $p < 0.01$; ns, not significant. (e) HE and (f) safranin O/fast green staining were used for morphological analysis of the modeled IVD. NP = nucleus pulposus; AF = annulus fibrosus; CEP = cartilage endplate; BEP = bone endplate; GP = growth plate. Immunofluorescence staining of (g) collagen II and (h) TNF- α was used to characterize infected IVD tissue sections at week 12. All results are representative data generated from at least three independent experiments. Data are presented as mean $\pm$ SD. The one-way ANOVA with the Tukey's multiple comparison test (d) was used for statistical analysis.

Po4_1.02

Can web-based applications improve visualisation and interpretation of machine learning predictions in Orthopaedics?

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Abstract

Introduction. Web-based applications are emerging as valuable platforms for deploying machine learning (ML) models in healthcare, offering transparent and interpretable predictions that could enhance clinicians' confidence in the ML-generated insights and promote adoption in practice. In musculoskeletal care, such tools may improve timely decision-making, supporting personalised management and resource allocation.

Aims. We aim to establish the utility of applications for visualising ML predictions to support clinical decision-making. We present two prototype applications: one predicts rapid knee osteoarthritis (OA) progression, while the other screens for significant soft tissue knee injuries (STKIs). These tools are designed to improve clinicians' engagement with predictive outputs and foster trust in ML-driven insights.

Methods. In the first study, an ML tool was developed to predict rapid knee OA progression using clinical and imaging data from the FNIH OA Biomarker Consortium. The second study employed retrospective clinical data to develop an application for detecting significant STKIs. Both studies utilised the *AutoPrognosis 2.0* automated ML (autoML) framework for model development, and Kernel-SHAP for interpretability analysis. Deployed on the Streamlit platform, these applications offer interactive interfaces for clinicians to explore predictive results and key influencing factors.

Results. Both applications (*Figure 1*) demonstrate the effectiveness of interactive visualisation in presenting ML predictions. In the knee OA app (https://autop-knee-oa-multiclass.streamlit.app/?embed_options=light_theme), users can explore the robustly validated predictive outputs (area and the precision-recall curve, or AUC-PRC 0.727, 95% CI: 0.726–0.728, *Table 1*) through an intuitive interface, examining how variations in patient characteristics influenced rapid OA progression. The STKI app (https://camkit-ai.streamlit.app/?embed_options=light_theme) offers similarly strong performance (AUC-PRC 0.849, 95% CI: 0.844–0.854, as shown in *Table 1*) and provides interactive visualisations of key predictive features, including instability, swelling, and weight-bearing status. By integrating Kernel-SHAP interpretability analysis, both applications promote transparency and provide a clearer understanding of clinical predictors.

Conclusions. These prototype applications demonstrate effectiveness of interactive visualisation in presenting predictions and suggest that interpretable ML models integrated

with interactive web platforms have the potential to significantly enhance clinical decision-making. Although further clinical testing is warranted, their interactive nature holds promise for increasing clinician engagement and understanding. By providing transparent, user-friendly interfaces, these tools could support personalised patient management and better resource allocation, especially in musculoskeletal care where early and precise interventions are crucial.

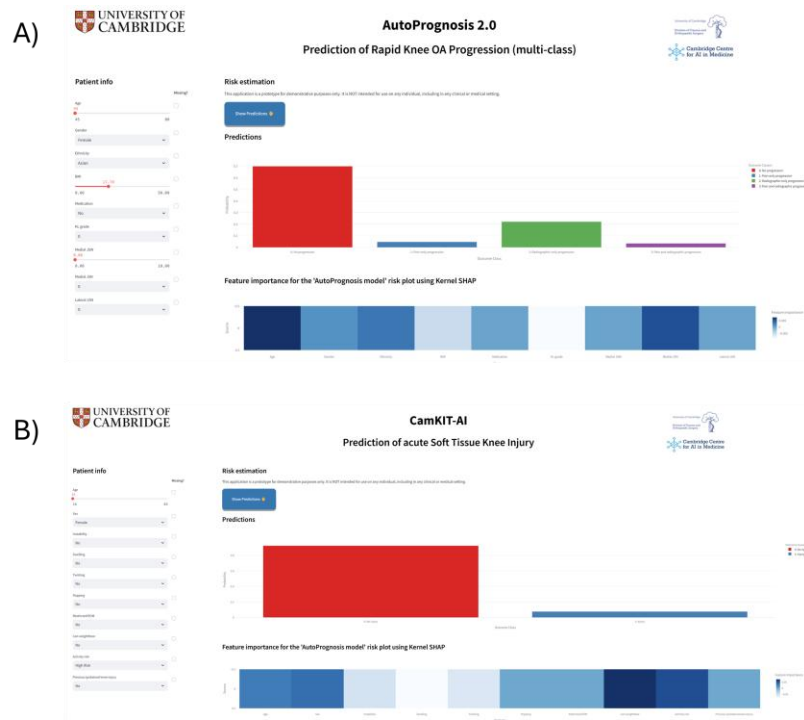


Figure 1. Apps for knee OA prediction (A) and STKI screening (B)

Model	AUC-PRC (95% CI)	AUC-ROC (95% CI)	F1 score (95% CI)
Knee OA prediction	0.727 (0.726-0.728)	0.881 (0.881-0.881)	0.593 (0.592-0.594)
STKI screening	0.849 (0.844-0.854)	0.928 (0.918-0.938)	0.839 (0.832-0.846)

Table 1. Models' performance

Po4_1.03

Evaluating the Reliability of AI-Based Long Leg Alignment Analysis in EOS Imaging and Severe Deformities

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Abstract

Background: Artificial intelligence (AI) is making its mark in many orthopaedic applications, including long-leg alignment analysis due to its time-saving capabilities and consistency. Previous studies have demonstrated high accuracy of AI tools when compared to manual readings. This study aims to examine the reliability of a leading software using deep learning algorithms (LAMA™, Image Biopsy Lab GmbH, Vienna, Austria) when applied to alternative imaging modalities (EOS) and a dataset containing severe lower limb deformities.

Methods: EOS images from 20 patients with varying degrees of lower limb deformities were analyzed. Two readers, an orthopaedic surgeon and a trained professional, performed manual measurements twice, with a two-week interval. The same dataset was analyzed using the AI tool. The agreement was evaluated for nine alignment parameters, including Mechanical Axis Deviation (MAD), Femur Length, Tibia Length, Leg Length, Joint Line Convergence Angle (JLCA), Mechanical Lateral Proximal Femoral Angle (mLPFA), Mechanical Lateral Distal Femoral Angle (mLDFA), Mechanical Medial Proximal Tibial Angle (mMPTA), and Mechanical Lateral Distal Tibial Angle (mLDTA). Intra-class correlation coefficients (ICCs) were used to assess inter-rater reliability, intra-rater reliability, and AI-manual comparisons. Bland–Altman plots were generated to evaluate the bias between manual readings and AI-generated results.

Results: The AI system was unable to generate results in 25% of cases, particularly in those involving implants or severe deformities. The comparison between AI and manual measurements, illustrated by the red points in Figure 1, revealed variability in reliability across different parameters. Angular measurements exhibited moderate agreement between the AI and manual methods (ICC ~0.30–0.70), while length measurements demonstrated strong agreement (ICC ~0.97–0.99). Additionally, inter-rater and intra-rater reliability analyses among manual readers showed high consistency across most measurements, as indicated by the blue points in Figure 1 (ICC > 0.97).

Conclusion: Although the AI tool shows promise for long-leg alignment analysis, its performance is limited in cases involving severe deformities and alternative imaging modalities like EOS. A significant failure rate and inconsistent accuracy in angular measurements highlight the need for further refinement of AI algorithms, incorporating a wider range of data types and imaging modalities.

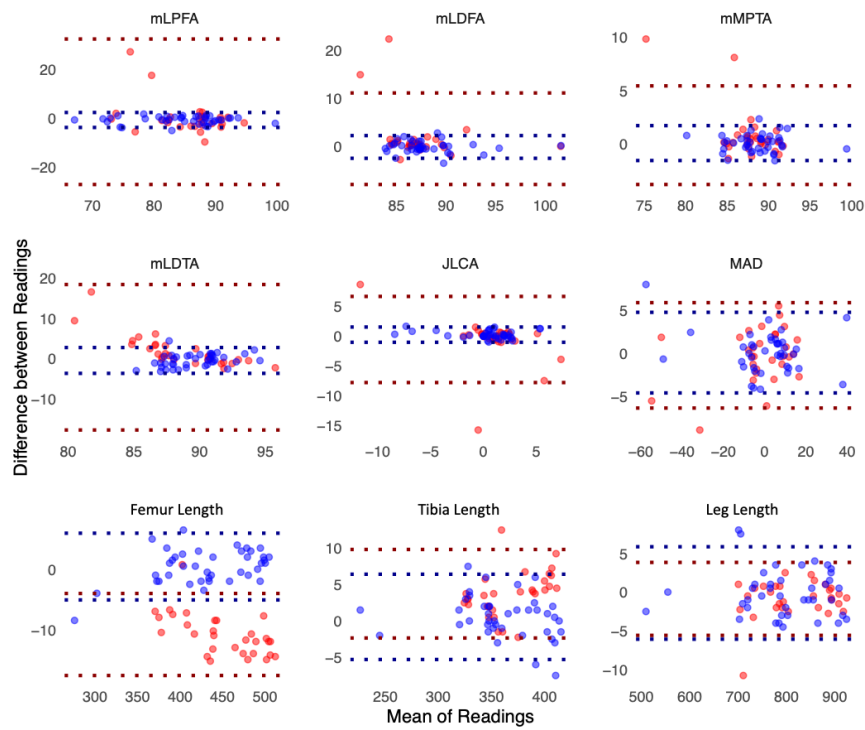


Figure 1. Bland-Altman plots illustrate agreement between AI-generated and manual measurements (red points) and consistency of inter-rater and intra-rater manual measurements (blue points).

Po4_1.04

Automated Radiographic Assessment of Lower Limb and Foot Alignment Using Deep Neural Networks

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Abstract

Background. Accurate radiographic assessment of lower limb alignment is crucial for surgical planning and outcomes. Current manual evaluations by specialists are resource-intensive and prone to inter-observer variability. We developed a deep neural network model to automate the measurement of lower limb alignment parameters in plane radiographs, aiming for comparable accuracy to existing approaches while addressing data limitations in clinical settings.

Methods. Ground truth data were generated through manual annotation of radiographs using a custom-built annotation tool in Python. The tool's modular design enables adaptation to various anatomical regions and provides guidance to the annotator to limit human errors. For automated annotation the machine learning algorithm was based on a stacked hourglass structure for precise landmark prediction. A two-stage approach was implemented: first, to define joint-specific regions of interest, and second, to localize anatomical landmarks within that region (Figure 1). The model was applied to two use cases: Ten alignment parameters from long-leg X-rays; and the hallux valgus angle (HVA) and intermetatarsal angle (IMA) in dorsoplantar foot X-rays.

For long-leg radiographs, the model was trained on 173 images, validated on 43 images, and tested on an external dataset. For foot angles, training utilized 243 radiographs, with 38 for validation and 20 for testing. Data augmentation and consistency strategies were applied to address small dataset sizes. Validation metrics included intraclass correlation coefficients (ICCs) for reliability assessment.

Results. The model achieved excellent agreement, with ICCs > 0.90 for nine of ten long-leg parameters in the test dataset. A more challenging dataset, including paediatric images, yielded ICCs > 0.76 for the three available parameters. For foot X-rays, two training approaches—landmark and axis methods—were evaluated. The axis method outperformed the landmark method, achieving ICCs of 0.96 and 0.83 for HVA and IMA, respectively.

Conclusion. The proposed model demonstrates high accuracy and reliability in automated lower limb alignment assessment, offering a robust solution for clinical settings with limited annotated datasets. Its modular and flexible architecture supports scalability and adaptation to other orthopedic applications, positioning it as a valuable tool for enhancing efficiency and precision in radiographic assessments.

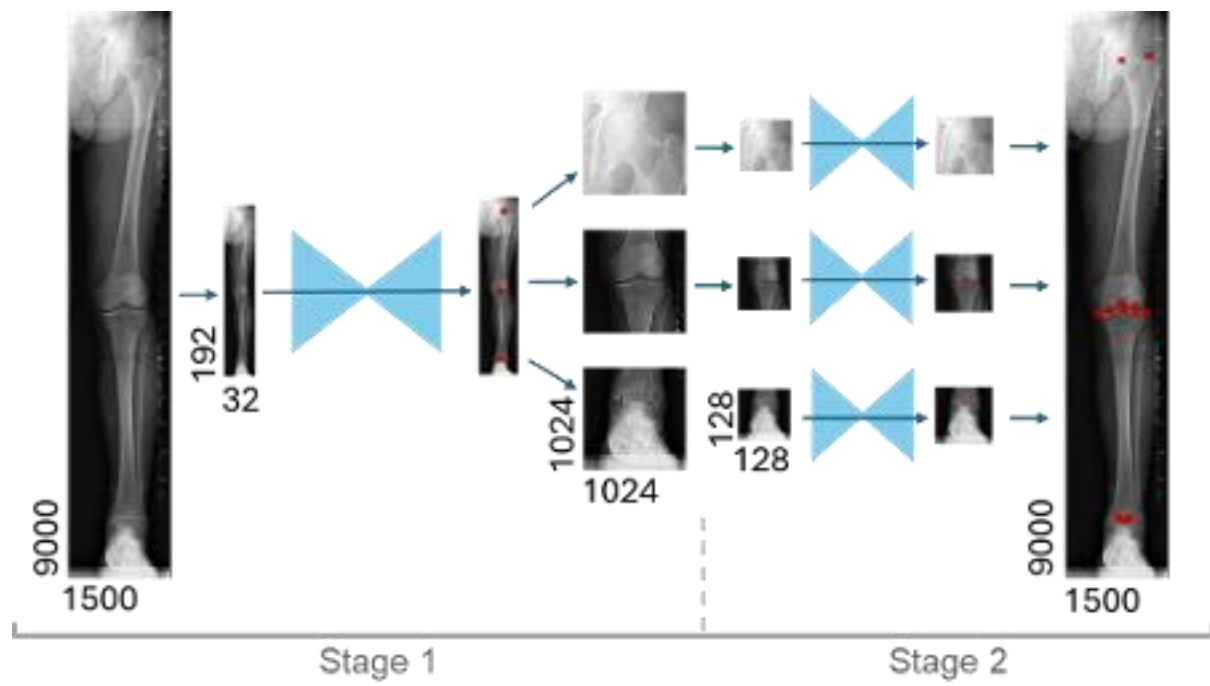


Figure 1: Two-stage approach for landmark prediction

Po4_1.08

AI-assisted diagnosis using DEANet to improve correct diagnosis of iliac wing fracture and ischial spine fracture

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Abstract

Rationale and Objectives. Pelvic fracture is precipitated by a high-energy impact such as a vehicular accident or a fall. There are numerous classifications of pelvic fractures, depending on the location of the fracture. The most common pelvic fractures, iliac wing, and ischial spine fractures require an accurate diagnosis for effective treatment planning. Traditional methods, relying on X-rays followed by CT or MRI, are time-consuming and prone to subjectivity. Therefore, the objective of this study is to develop a deep learning-based AI-assisted diagnostic method to reduce reliance on high-cost and high-risk imaging techniques such as CT and MRI. By analyzing radiographic features of iliac wing and ischial spine fractures, our approach seeks to minimize diagnostic delays, prevent patient deterioration, and enhance the accuracy of fracture identification.

Materials and Methods. In this study, we propose a deep learning-based two-branch binary classification model, DEANet, for the automatic classification of iliac wing fractures and ischial spine fractures. To enhance the features of the edge region and improve the classification of hip fractures from pelvic X-ray images (PXR), we employed an attention mechanism using the EDSA Block. The dataset was obtained from pelvic X-ray image data of China Medical University. To evaluate the performance of the model, a series of metrics were employed, including accuracy, precision, recall, F1 score, and IoU.

Results. The dual-branch classification model demonstrated an 85% accuracy rate on the test dataset and exhibited strong performance on several evaluation metrics, including precision, recall, and F1 score. These findings indicate that the model is capable of effectively differentiating between iliac wing and ischial spine fractures, providing a valuable reference for the diagnosis of iliac fractures.

Conclusion. The proposed deep learning model of DEANet demonstrates considerable promise and efficacy in hip fracture classification. The findings indicate that this AI-assisted diagnostic instrument can enhance the precision and reliability of clinical diagnosis, offering medical professionals a valuable resource for more accurate surgical planning and treatment plan selection.

Po4_1.09

Deep Learning-Based Volumetric Analysis and Steinberg Classification of Osteonecrosis of the Femoral Head

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Abstract

Background: Volumetric assessment of necrotic lesions using the Steinberg classification is a valuable predictor of future collapse in osteonecrosis of the femoral head (ONFH). However, the manual quantification of these lesions using magnetic resonance imaging (MRI) is labor-intensive, limiting the routine clinical application of the Steinberg classification. This study aimed to develop a deep learning-based method for automatically segmenting necrotic lesions on MRI and classifying them according to the Steinberg classification.

Methods: A total of 63 hips from patients with ONFH without collapse were included. An orthopedic surgeon manually segmented the femoral head and necrotic lesions on MRI acquired using a spoiled gradient-echo sequence. Based on these manual segmentations, the hips were classified into Steinberg grades: 22 hips as grade A, 23 as grade B, and 18 as grade C. The manually segmented labels were used to train a deep learning model that used a 5-layer Dynamic U-net system. Four-fold cross-validation was performed to assess segmentation accuracy using the Dice coefficient (DC) and average symmetric distance (ASD). Furthermore, hip classification accuracy according to the Steinberg classification was evaluated along with the weighted Kappa coefficient.

Results: The median DC and ASD for the femoral head region were 0.954 (interquartile range [IQR], 0.947–0.961) and 0.649 mm (IQR, 0.589–0.754 mm), respectively. For necrotic lesions, the median DC and ASD were 0.890 (IQR, 0.846–0.918) and 0.758 mm (IQR, 0.578–0.960mm), respectively. A strong correlation ($r = 0.950$) was observed between the percent volume of necrotic lesions assessed by the deep learning model and manual segmentation (Fig. 1a). The deep learning model correctly classified Steinberg grades in 59 of 63 hips (accuracy: 93.7%), with a weighted Kappa coefficient of 0.979 (Fig. 1b).

Conclusions: The proposed deep learning model demonstrated high accuracy in segmenting necrotic lesions and classifying them according to the Steinberg classification using MRI. This model has the potential to assist clinicians in efficiently performing volumetric assessments of ONFH.

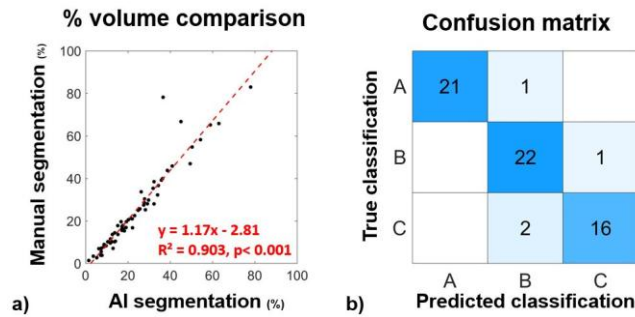


Figure 1. Correlation plot of the percent of necrotic lesions within the head region (a) and the confusion matrix of the Steinberg classification (b) when the segmentation was performed manually or by the deep learning model. Each number in (b) indicates the number of cases included in each category.

Po4_1.10

Revolutionizing Orthopaedics: Opportunities and Challenges of Code-Free Deep Learning Technologies

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Abstract

The emergence of code-free deep learning platforms has significantly impacted various domains, including orthopaedics, by broadening access to sophisticated artificial intelligence methodologies. This review article examines the uses, opportunities, challenges, and future perspectives of code-free deep learning in orthopaedics, emphasizing both its potential to innovate and the obstacles it faces.

In the field of orthopaedics, code-free deep learning tools empower healthcare professionals and researchers to analyze intricate datasets, such as medical images and patient histories, without the need for programming expertise. Key applications include automated image analysis, fracture classification, and outcome prediction, all of which contribute to improved diagnostic precision and the establishment of personalized treatment regimens. These platforms further streamline the creation of predictive models, leading to enhanced patient management through timely interventions and customized therapies.

However, the broad adoption of code-free deep learning in orthopaedics is challenged by several factors. Primarily, the availability and quality of training data pose significant issues, as the effectiveness of machine learning algorithms hinges on robust datasets. Frequently, medical imaging datasets may be limited or exhibit imbalances that hamper the generalizability of the developed models across diverse patient cohorts. Additionally, concerns regarding data privacy and security arise, necessitating that patient information be handled in accordance with regulations, such as HIPAA.

Another critical challenge is the interpretability of models generated through code-free platforms. While the simplification of the model-building process is advantageous, it can lead to a lack of transparency regarding predictive mechanisms, which may diminish clinicians' confidence in utilizing the outcomes effectively. Thus, establishing collaborative efforts between data scientists and orthopaedic experts is vital for ensuring that models remain relevant in clinical settings and are understood thoroughly.

Looking towards the future, the prospects of code-free deep learning in orthopaedics appear promising. Emerging trends indicate potential integration with patient management systems and electronic health records, promoting more efficient workflows. Moreover, advancements in techniques like transfer learning and few-shot learning are poised to

mitigate the limitations related to data scarcity, enabling practitioners to apply existing models to specialized contexts.

In summary, while code-free deep learning presents significant opportunities to revolutionize orthopaedic practice, addressing data quality, interpretability, and regulatory challenges is essential. By fostering interdisciplinary partnerships and refining these technologies, the orthopaedic field can leverage AI's capabilities to improve patient care, enhance surgical effectiveness, and explore innovative therapeutic avenues.

Po4_1.11

Foundation Models: Shaping the Future of AI in Real-World Orthopaedic Practice

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Abstract

Foundation models, such as large-scale pre-trained neural networks, are transforming various sectors, including healthcare, with profound implications for orthopaedics. These advanced models utilize extensive datasets and sophisticated architectures to enhance clinical decision-making across multiple orthopaedic applications. This review aims to examine the current utilization of foundation models in orthopaedics, the opportunities they embody, the challenges they face, and potential future directions for both research and clinical implementation.

In the realm of orthopaedics, foundation models hold promise in several significant areas: diagnostic imaging, predictive analytics, robotic-assisted surgery, and the development of personalized treatment strategies. Specifically, in diagnostic imaging, models trained on large datasets of radiographs and MRI scans can significantly improve the accuracy of detecting and classifying fractures, thereby facilitating prompt and effective treatments. Furthermore, the implementation of natural language processing (NLP) within these models enables the extraction and analysis of clinical notes and relevant literature, enhancing decision support systems for healthcare providers.

Predictive analytics driven by foundation models can help assess patient outcomes by analyzing historical healthcare data, allowing orthopaedic surgeons to anticipate complications and refine preoperative strategies. In robotic-assisted surgery, these models can be integrated into surgical systems, improving the precision and adaptability of procedures. Additionally, personalized treatment plans can be optimized through foundation models by analyzing a combination of genetic, environmental, and clinical data, leading to improved patient outcomes through tailored approaches.

Nonetheless, the integration of foundation models in orthopaedics presents significant challenges. Issues regarding data privacy, the representativeness of training datasets, and the risk of introducing bias into clinical decisions are critical concerns that require careful attention. Moreover, addressing the opaque nature of these models is vital, as understanding their decision-making processes is essential for fostering trust and transparency within the clinical community.

Looking forward, future pathways for implementing foundation models in orthopaedics include enhancing model interpretability, establishing comprehensive regulatory frameworks, and promoting interdisciplinary collaborations. Further research is needed to

develop models that are efficient, equitable, and accessible across diverse patient populations. Utilizing real-world data can also help validate model performance, while ongoing education and training for healthcare professionals in AI technologies will facilitate their effective integration into clinical workflows.

In summary, foundation models present remarkable opportunities to enhance orthopaedic practice, but their successful implementation must address ethical and practical challenges. Taking proactive steps to tackle these issues will be key to fully realizing their potential in the future.

Po4_1.12

Orthopaedics-grounded generative adversarial network: friend or foe?

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Abstract

Generative Adversarial Networks (GANs) represent a transformative innovation in artificial intelligence, offering significant advantages in the domain of orthopaedics. This review article explores the diverse applications of GANs in orthopaedics, emphasizing their role in enhancing medical imaging, developing patient-specific models, and personalizing treatment strategies. The unique architecture of GANs, comprising two adversarial networks—the generator and the discriminator—enables the synthesis of high-quality synthetic data. This capability is crucial for overcoming data limitations and improving the training of machine learning models within the field.

One of the key applications of GANs in orthopaedics lies in the improvement of medical imaging techniques. By producing realistic synthetic images for training purposes, GANs enhance the precision of diagnostic models, particularly in the identification of fractures, joint disorders, and tumors. Furthermore, GANs can facilitate the transformation of images across different modalities, such as converting MRI scans into CT images, thus enabling comprehensive evaluations and more effective treatment planning.

Additionally, GANs play a vital role in generating patient-specific anatomical models. By leveraging individual patient data to create customized representations, GANs aid orthopaedic surgeons in preparing for intricate procedures, such as joint replacements or corrective surgeries. These tailored models enhance preoperative planning accuracy, reduce surgical complications, and contribute to improved patient outcomes by optimizing intervention strategies.

Moreover, GANs are advancing personalized medicine in orthopaedics by supporting the design of individualized treatment plans. By analyzing patient data and generating synthetic clinical scenarios, GANs assist healthcare providers in predicting the effectiveness of various treatment options, thus fostering more targeted and efficient care strategies.

Despite the numerous benefits associated with GANs in orthopaedics, several challenges persist. Issues such as the requirement for high-quality training datasets, the potential for model overfitting, and concerns regarding the interpretability of generated outputs represent significant barriers that must be addressed to fully harness the capabilities of GANs in clinical settings.

In summary, the integration of Generative Adversarial Networks within orthopaedic practice holds tremendous potential for advancing the field by enhancing imaging techniques,

facilitating personalized models, and enabling tailored treatment approaches. Ongoing research and collaboration between data scientists and orthopaedic practitioners are crucial for overcoming existing challenges and maximizing the transformative impact of GANs on patient care and outcomes.

Po4_1.13

Utilizing Machine Learning to Enhance Core Outcome Set Development: Automating the Extraction and Classification of Outcomes

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Abstract

Background: Core Outcome Sets (COS) are essential for standardizing outcome reporting in clinical and research settings, promoting consistency and comparability across studies. They support evidence synthesis and inform clinical decision-making. However, manually extracting and categorizing outcomes from the literature is resource-intensive, time-consuming, and prone to variability. This study investigates the potential of machine learning to automate these processes, aiming to enhance efficiency, consistency, and scalability in outcome identification and classification.

Methods: 114 articles were used to assess the feasibility of training a model to identify verbatim outcomes and determine the number of articles needed for acceptable performance. A total of 2,941 verbatim outcomes were manually extracted from the "Results" sections, while verbatim non-outcomes, defined as noun phrases with modifiers, were randomly sampled from sections preceding the "Results."

Using fine-tuning of an existing language model, predictive models were trained iteratively in increments of five articles. Each iteration used a balanced dataset of outcomes and non-outcomes, split 80-20 into training and test sets. Performance was evaluated on a consistent hold-out set of 28 articles.

A second model was developed to classify outcomes into predefined COMET taxonomy domains, using the suggested number of articles. Precision, recall, and F1-scores were calculated for both tasks, with stratified k-fold cross-validation ensuring robustness and reducing overfitting.

Results: For predicting verbatim outcomes, results indicate that a model based on just 20 articles can achieve acceptable model performance. The final model trained on 20 articles achieved a precision of 94.3%, recall of 95.3%, and an F1-score of 94.8%. When applied to the full dataset of 114 articles, the model correctly identified 94.3% of verbatim outcomes. A 5-fold cross-validation for the 20-article model yielded an average precision of 94.8%, recall of 96.8%, and F1-score of 95.7%.

The second model for outcome domain classification achieved a weighted F1-score of 90.2%. High predictive accuracy was observed for domains such as musculoskeletal and connective tissue outcomes (99.2%) and adverse events (93.1%). Lower performance was

noted in underrepresented domains, such as role functioning and social functioning, which had limited training examples.

Conclusion: This study demonstrates the feasibility of using AI to automate outcome extraction and classification for COS development. By reducing manual effort and enhancing reliability, these methods support systematic reviews, scoping reviews, and consensus processes. AI's capability to process large-scale verbatim data with high precision highlights its transformative potential in advancing evidence-based clinical practice through more efficient and standardized outcome reporting.

Po4_1.15

Deep learning model for differentiating acute and chronic osteoporotic vertebral compressive fracture from multidetector CT

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Abstract

Objective: To develop a deep learning model capable of distinguishing between fresh and old osteoporotic vertebral compression fractures (OVCFs) on CT images.

Methods: A retrospective study was conducted, including 10,199 CT transverse images from 400 OVCF patients who underwent both vertebral CT and MRI examinations (with an interval of no more than 1 month between the two examinations) at the Second Affiliated Hospital of Nanjing Medical University from January 1, 2020, to November 1, 2023. The images were randomly divided into a training set of 8,629 images (84.6%) and an internal test set of 1,570 images (15.4%). Additionally, 1,158 CT transverse images from 70 OVCF patients who underwent the same examinations at Jiangsu Provincial People's Hospital from January 1, 2023, to November 1, 2023, were included as an external test set. Three doctors annotated the regions of interest of the fractured vertebrae using rectangular boxes in Anaconda Prompt software. A deep convolutional neural network (CNN) was trained on the annotated training set. The CNN with the best performance on both the internal and external test sets was selected as the final model and compared with the diagnostic abilities of three orthopedic doctors.

Results: In the internal test set of 1,570 images, the model achieved an AUC of 0.92 (95% CI: 0.89, 0.94), a sensitivity of 93.3%, and a specificity of 88.3%. In the external test set of 1,158 images, the model's AUC was 0.88 (95% CI: 0.77, 0.92), with a sensitivity of 86.3% and a specificity of 81.8%. The model outperformed the best-performing orthopedic doctor in both the internal (AUC: 0.86 [95% CI: 0.78, 0.91]; $P < 0.001$) and external (AUC: 0.71 [95% CI: 0.60, 0.82]; $P < 0.001$) test sets.

Conclusion: The developed deep learning model demonstrated a high capability in distinguishing between fresh and old OVCFs, surpassing the performance of orthopedic doctors.

Po4_1.16

Automated Prediction of Bone Mineral Density and T-Score from Radiographs via Deep Learning

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Abstract

Background. Osteoporosis is a prevalent bone disease characterized by reduced bone mineral density (BMD) and increased fracture risk. Accurate assessment of BMD and T-scores is crucial for early detection, diagnosis, and treatment of osteoporosis. Traditional methods for BMD estimation involve dual-energy X-ray absorptiometry (DXA), which requires specialized equipment and exposes patients to ionizing radiation.

Methods. This study proposes a deep learning framework for BMD and T-score prediction using radiographs. EfficientNetB7 was used for training, validating and testing of datasets and subsequently rendered simulated T-scores for classification of osteoporosis. The algorithm was developed under the Python environment. To assess the performance of the EfficientNetB7, the multi-class confusion matrix was employed and was used to produce five major assessment parameters – accuracy (ACC), precision (P), sensitivity (Sn), specificity (SP) and F-score values. Pearson's correlation coefficient and Mean Absolute Error (MAE) were utilized to investigate the statistical relationship between the simulated T-scores and DXA T-scores.

Results. A total of 5,000 images were included in the study. We explored the application of combined supervised and unsupervised learning models for predicting BMD and T-scores from radiographs, providing a non-invasive and cost-effective alternative to DXA. The correlation coefficients for BMD prediction were 0.96, the MAE for T-score prediction was 0.6.

Conclusion. Our findings demonstrated that the deep learning model achieves promising performance in predicting BMD and T-scores from radiographs, with MAE value comparable to or better than traditional DXA-based methods. The correlations between predicted and actual BMD/T-scores indicate a strong linear relationship, further validating the effectiveness of the proposed approach.

Po4_1.17

Effectiveness of a Large Language Model in Reverse Shoulder Surgery

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Abstract

The integration of Large Language Models (LLMs) in healthcare has demonstrated significant potential to enhance clinical outcomes and patient engagement. This study focuses on the application of LLMs in reverse shoulder surgery, a field where the complexity of patient data and the need for precision in surgical decision-making are critical. By employing a mixed-methods approach that combines a comprehensive literature review with an analysis of current practices, this research identifies gaps in the application of LLMs within orthopedic surgery. Quantitative analysis of surgical outcomes, integrated with qualitative assessments of patient experiences, evaluates the effectiveness of LLM tools in improving communication between healthcare providers and patients, streamlining clinical documentation, and enhancing preoperative education. Preliminary findings indicate that LLMs significantly enhance patient comprehension of surgical procedures, facilitating more informed decision-making processes. Trials utilizing bone models confirmed the surgical assistant's accuracy, efficiency, and practical applicability, reinforcing its potential utility in real-world surgical settings. The implementation of LLM tools not only improved communication and clinical workflows but also increased patient engagement and overall satisfaction. Specifically, the research highlights the transformative potential of LLMs in reverse shoulder surgery, offering valuable insights to optimize patient care and refine clinical practices. These results underscore the need for continued investigation into the broader application of AI technologies, particularly in orthopedic surgery, to further validate and expand upon these promising findings. This study advocates for the incorporation of advanced LLM tools in healthcare systems to optimize surgical outcomes and patient satisfaction, with implications for health policy, especially in regions like Hong Kong, where such innovations can drive improvements in patient care and clinical efficiency. Future research should explore broader applications of LLMs in healthcare to maximize their potential benefits.

Po4_1.18

Diagnosis of Femoral Head Necrosis and Three-dimensional Spatial Localization of Necrotic Lesions Based on CT Perfusion Imaging Technology

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Abstract

Objective: Traditional CT or MRI examinations are difficult to simultaneously achieve early diagnosis and accurate localization of femoral head necrosis, which makes it challenging to further predict the risk of femoral head collapse, which has a certain impact on the effectiveness of early hip preservation treatment. This study aims to achieve rapid diagnosis of femoral head necrosis and fully automated 3D reconstruction of the necrotic area of the femoral head based on artificial intelligence (AI) and CT perfusion imaging technology, making it possible to accurately localize the femoral head necrotic area. **Methods:** This study selected 85 patients with pre-stage IIIA femoral head necrosis according to the ARCO staging system and collected preoperative CT perfusion imaging and MRI data. Based on the CT-MRI rigid image registration technique using mutual information (MI), MRI was used as the gold standard to segment the femoral head necrotic area and train the U-Net model based on CT perfusion imaging to achieve automatic segmentation of the necrotic lesion of the femoral head. We studied the sensitivity of various perfusion parameters on femoral head necrosis through a multimodal fusion model and evaluated the accuracy of image registration and model segmentation using the Target Registration Error (TRE) and dice similarity coefficient (DSC). **Results:** (1) Rigid multimodal image registration based on MI can achieve high-precision hip joint CT-MRI image registration ($TRE=3.071\pm0.932$ mm). (2) The deep learning model had a high DSC coefficient for MSI ($DSC = 0.917\pm0.105$), BF ($DSC = 0.941\pm0.092$), and BV ($DSC = 0.924\pm0.079$). (3) The fusion model of multiple perfusion parameters obtained higher DSC values, and the combination of Average + BF + TTP + MTT + BV achieved the highest DSC (0.954 ± 0.072). (4) The 3D reconstruction model of femoral head necrosis based on the best fusion model was highly similar to the MRI manual annotation results ($DSC= 0.944\pm0.112$). **Conclusion:** The deep learning model based on CT perfusion imaging can rapidly diagnose early femoral head necrosis and accurately reconstruct the 3D structure of femoral head necrotic lesions.

Po4_2.01

The Impact of Bilateral Total Hip Arthroplasty on Lower Extremity Biomechanics in Bilateral Developmental Dysplasia of the Hip-Associated Secondary Coxarthrosis: Medium-to-Long-Term Valgus Alignment in the Knee Joint

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Abstract

This study aimed to evaluate the impact of THA on lower extremity biomechanics, coronal plane alignment, and knee joint changes during medium-to-long-term follow-up in patients who underwent bilateral THA at different time points for DDH-associated secondary coxarthrosis.

A total of 17 patients (1 male, 16 female) with 34 hips who underwent staged bilateral THA were included in the study. The mean age of the patients was 44.7 years, with an average follow-up period of 49.6 months. The mean interval between the two surgeries was 9.3 months. Patients were categorized based on the Crowe classification, and the types of femoral stems utilized were recorded. Standardized measurements were performed on preoperative and final follow-up leg-length radiographs. Pre- and postoperative knee Kellgren-Lawrence (KL) grades, mechanical and anatomical lateral distal femoral angles (mLDFA, aLDFA), Q angles, hip-knee-ankle (HKA) angles, femur mechanical-anatomical angle (VKA), tibiotalar (TT) angle, mechanical medial proximal tibial angle (mMPTA), joint line congruence angle (JLCA), and knee mechanical axis deviation (MAD) ratios were compared. Statistical analysis was conducted using IBM SPSS 24.0, and sample size was determined through power analysis.

Among the hips, 8 were classified as Crowe 1-2 and 26 as Crowe 3-4. Femoral stem types included conical in 9 hips, cylindrical in 7 hips, and rectangular in 18 hips. No significant differences were observed in mLDFA, aLDFA, Q angle, HKA angle, VKA, or TT angle between preoperative and postoperative evaluations. However, postoperative comparisons indicated a valgus tendency in HKA, VKA, and TT angles relative to preoperative values. The type of femoral stem did not significantly affect postoperative measurement outcomes. Nevertheless, significant increases were found in postoperative mMPTA ($p = 0.015$), JLCA ($p = 0.009$), MAD ratio ($p = 0.007$), and KL grades ($p < 0.0001$). Notably, a valgus inclination was identified in the same-sided knees. In hips classified as Crowe 3-4, differences in JLCA and KL grade between pre- and postoperative evaluations were significantly greater compared to Crowe 1-2 hips ($p = 0.014$, $p = 0.001$, respectively).

These findings indicate a medium-to-long-term valgus tendency in the lower extremity and knee joint following THA, which may contribute to gonarthrosis. This valgus tendency was particularly pronounced in Crowe 3-4 hips. While previous studies have predominantly focused on the effects of unilateral DDH and THA on the knee, this study highlights that bilateral DDH and THA exacerbate valgus alignment in the knee. Future studies should further explore the clinical implications of this phenomenon.

Po4_2.02

The Impact of Total Hip Arthroplasty on Lower Extremity Biomechanics in Unilateral Developmental Dysplasia of the Hip-Associated Secondary Coxarthrosis: The Course of Valgus Alignment in the Knee Joint

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Abstract

This study aimed to evaluate the medium-to-long-term effects of unilateral THA performed for secondary coxarthrosis due to unilateral DDH on lower extremity biomechanics, coronal plane alignment, knee joint parameters and the course of valgus alignment in the knee joint.

A total of 74 patients (15 males, 59 females) with unilateral THA (74 hips) were included. The mean patient age was 44.6 years, with an average follow-up period of 45.42 months. Based on the Crowe classification, 49 hips were categorized as Crowe 1-2 (Group A) and 25 hips as Crowe 3-4 (Group B). Femoral stem types used included 13 conical, 19 cylindrical, and 40 rectangular designs. Measurements were performed using preoperative and final follow-up long-leg radiographs. Parameters analyzed included pre- and postoperative knee Kellgren-Lawrence (KL) grades, mechanical and anatomical lateral distal femoral angles (mLDFA, aLDFA), pelvic tilt, Q angle, hip-knee-ankle (HKA) angle, femur mechanical-anatomical angle (VKA), tibiotalar (TT) angle, mechanical medial proximal tibial angle (mMPTA), joint line congruence angle (JLCA), and knee mechanical axis deviation (MAD) ratios. Data were analyzed using IBM SPSS 24.0, and sample size was determined via power analysis.

In Group B, postoperative mMPTA ($p=0.016$), pelvic tilt ($p=0.012$), postoperative leg length discrepancy ($p=0.027$), Q angle ($p=0.018$), HKA angle ($p=0.002$), and knee MAD ($p=0.029$) were significantly higher, while postoperative VKA ($p=0.001$) was significantly lower compared to Group A. No significant differences were found between the groups for other parameters. In Group B, postoperative JLCA, Q angle, HKA angle, TT angle, and knee MAD increased, while in Group A, Q angle, HKA angle, TT angle, and knee MAD decreased. Postoperative VKA decreased in Group B but increased in Group A. In Group B, valgus alignment in the knee joint increased postoperatively compared to preoperative values, whereas in Group A, valgus alignment decreased. In both groups, postoperative KL grades showed a significant increase ($p<0.0001$). Among patients with conical femoral stems, postoperative VKA and mLDFA values were significantly lower than in other stem types ($p=0.002$; $p=0.009$), and the reduction in VKA was significantly greater ($p=0.037$).

These findings indicate that in medium-to-long-term follow-up after THA, valgus alignment in the knee joint increased in Crowe 3-4 hips but improved in Crowe 1-2 hips, although this improvement did not prevent progression to gonarthrosis. Additionally, the use of conical femoral stems resulted in significant changes in the mechanical axis of the femur. Future studies should evaluate the clinical implications of these findings in comparative settings.

Po4_2.03

Regulatory Network Modeling of Macrophage Polarization Signaling Pathways in the Synovium of the Normal Knee Joint: Establishing a Baseline for Subsequent Modeling of Osteoarthritic Knee Joint Dynamics

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Abstract

Macrophages are central to OA-related inflammation in the synovium and play a critical role in disease progression. They dynamically shift between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, governed by complex regulatory networks. As mechanistic understanding of such complexity can be instrumental for the development of disease-modifying osteoarthritis treatments, this study aims to construct a semi-quantitative regulatory network model of macrophage polarization within the knee joint synovial microenvironment.

A systematic regulatory network modelling approach was employed to map key signaling pathways influencing macrophage polarization. An extensive literature review and manual curation of the retrieved information led to a corpus of 100+ peer-reviewed journal articles that were used to define a directed interactome of proteins, metabolites and substrates involved in macrophage activity and polarization. The model incorporated 90 nodes, linked among each other by 270, either inhibiting or activating, links. The nodes consisted in transcription factors, cytokines, and metabolic sensors, the differential activation of which allows to characterize the dynamic balance between M1 and M2 states. Node activations were calculated on a continuous normalized scale, by solving mathematically the interactions, according to a fuzzy logic analogical interpolation of Boolean rules [*Mendoza L, Xenarios I*]. Network stability was assessed through a baseline analysis that consisted in 500 simulations with initial random activations of the nodes.

Baseline network simulation converged towards a stable state that predominantly supports the anti-inflammatory M2 phenotype (Fig.1A). Most anti-inflammatory markers (e.g. IL4, IL13, IL10, STAT3, STAT6, AMPK, Nrf2...) were up regulated while most pro-inflammatory markers (e.g. TNF- α , IFN- γ , NF- κ B, iNOS...) were down regulated. The regulatory network highlights the importance of metabolic gene expression, cytokine signaling, and transcriptional regulation in maintaining macrophage homeostasis. Network analysis revealed an overall decentralised network (Fig. 1A), where node connectivity and functional node-node interactions were well distributed (low centralization and clustering coefficients, relatively high network heterogeneity coefficient). These insights form the basis for the investigation of the dysregulation observed in OA, offering a way to shed light on the molecular mechanisms underlying M1/M2 balance in the synovium of knee joint.

Subsequent modelling in the osteoarthritic synovium and integration with computational chondrocyte models shall broaden the perspective on OA pathogenesis. Additionally, by identifying key regulatory nodes, this research shall support the exploration of biologic or local drug therapies that favour M2 polarization, aiming to reduce inflammation and improve clinical outcomes.

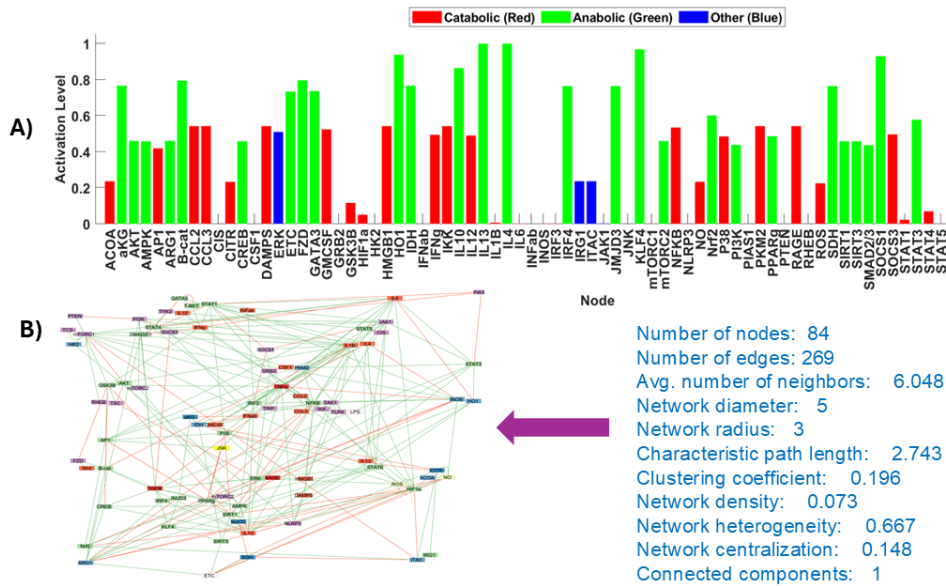


Figure 1. Mean Expression level of nodes(A) and static topology of interacting nodes(B)

Po4_2.04

An Exploration of Contact Pressure, Force, and Area Disparities in Balanced UKA: A Cadaveric Study Comparing Conventional and Robotic-Assisted Techniques

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Abstract

Introduction. Unicompartmental knee arthroplasty (UKA) offers advantages such as shorter recovery times and improved function compared to total knee arthroplasty (TKA). However, UKA has higher long-term revision rates, largely due to aseptic loosening and the progression of osteoarthritis. This study introduces automated procedures in flexion-extension to compare contact pressure, force, and area between conventional and robotic-assisted UKA in well-balanced knees, providing insights into biomechanical differences.

Methods. Sixteen fresh-frozen human cadaveric specimens with mild-to-moderate medial compartment osteoarthritis underwent UKA using conventional and CORI-assisted robotic techniques. A Tekscan sensor measured medial and lateral compartment contact pressures, areas, and forces from 90° flexion to full extension. Specimens were tested under a standardized extension rate (1.67°/s), and measurements were recorded for each compartment during three repetitions per technique. Data were analyzed for trends and statistical significance. Figure 1 and 2 illustrates the experimental set up.

Results. Medial compartment contact pressures progressively increased from 251.73 KPa at 90° to 1863.83 KPa at 0°, while lateral compartment pressures ranged from 319.83 KPa to 733.94 KPa. Statistically significant differences ($p < 0.05$) between compartments were noted at all angles except 90°. Lateral forces were consistently higher, with values at 0° reaching 97.84 N compared to 83.01 N medially. Lateral contact areas were significantly larger across all angles, peaking at 140.41 mm² (conventional) and 128.36 mm² (robotic) at 0°. Comparisons between conventional and robotic techniques showed no significant differences in pressure, force, or area ($p > 0.05$).

Conclusion. Both conventional and robotic-assisted unicompartmental knee arthroplasty (UKA) exhibited comparable biomechanical characteristics, yet the lateral compartment was consistently subjected to greater forces and larger contact areas. This significant difference in load distribution may play a role in the long-term degeneration of the joint, underscoring the critical need for precise balancing during the surgical procedure. While robotic assistance shows promise in enhancing load distribution, additional research is necessary to evaluate its long-term clinical benefits effectively. These findings offer vital insights into the optimization of surgical techniques and the design of implants for UKA, ultimately aiming to improve patient outcomes and prolong the health of the joint.

Figure 1: Experimental Set Up for 90-degree Flexion, TekScan film was put between the articulating surface of the femur and tibia.

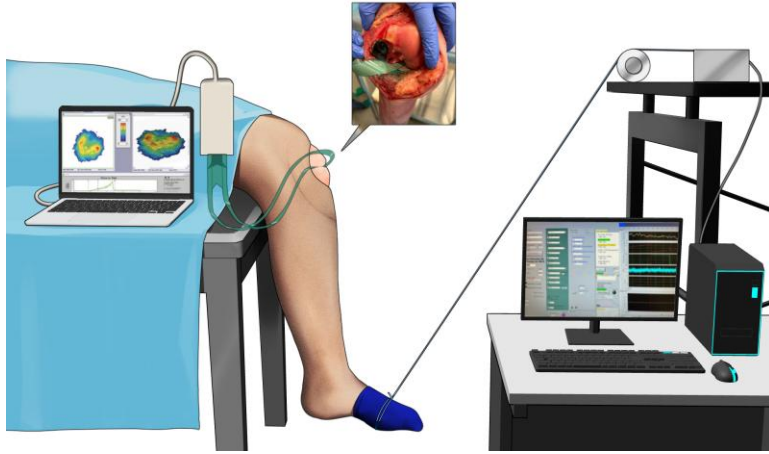
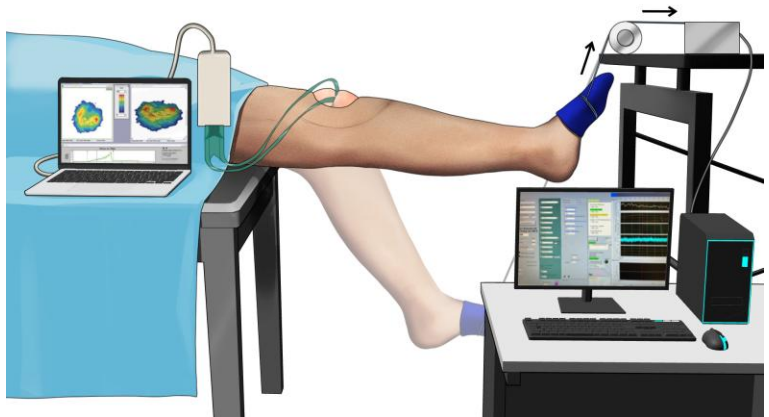


Figure 2: Experimental Set Up for 0-degree flexion (Complete Extension)



Po4_2.06

Combined clinical, biological and biomechanical investigations in the evaluation of knee osteotomy alone and combined with meniscal allograft transplantation.

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Abstract

Abnormal varus/valgus knee deformity can often occur in conjunction with meniscal deficit and results in compartment overload, gonarthrosis, altered locomotion and pain. In young and active patients, knee-osteotomy is performed to restore physiological knee alignment by preserving original knee anatomy. Despite good results, post-operative residual disability, dissatisfaction and pain are also reported. These could be overcome by additional meniscal-allograft-transplantation to address possible concomitant meniscal issues. This study's aim is to evaluate the effect of knee-osteotomy alone or combined with meniscal-allograft-transplantation using a novel investigative approach merging clinical, biomechanical, and biological evaluations. Fifty-two patients with, lower-limb malalignment and meniscal deficiency (graded ≤ 3 according to the Kellgren-Lawrence classification) were recruited for knee-osteotomy alone (26 patients) or combined with meniscal-allograft (26 patients); so far 9 and 7 patients were treated, respectively. Pre- and post-operative (at 12-month follow-up) examinations involved clinical assessments via scoring systems, biomarker assessments of cartilage turnover/inflammation (interleukin IL-8 and IL1 β), magnetic-resonance-imaging and functional evaluations via gait-analysis during daily-living activities, using a novel procedure to calculate the intersection of ground-reaction-force with patient-specific tibial-plateau morphology. To this, gait-analysis data and image-based morphological reconstruction were combined by means of additional bimodal 3D-printed reference markers placed around the tibia-plateau rim for registration purpose. Student's-t or Mann-Whitney-Wilcoxon test is used in data comparison, along with Pearson's-product-moment correlation. This study received institutional-review-board approval. Pre-operative examinations confirmed gonarthrosis, knee varus/valgus deformity (between 8°-valgus to 9°-varus), altered knee frontal/sagittal kinematics and kinetics, and inflammatory trend. Before surgery, patients with apparently mild meniscal deficit exhibited intersections outside the medial-lateral plateau-edge (~60% medial-to-lateral tibial-plateau width); with marked meniscal deficit these were outside the anterior plateau-edge (~55% anterior-to-posterior width). Post-op examinations revealed more physiological knee realignments, significant decreases in the original deformity ($R=-0.7$) and intersection dispersion ($R=-0.6$), and significant improvements in functional performances as per clinical scoring and gait

analysis (see figure), slightly better with meniscal-allograft. Post-op stable inflammation level was generally observed. This cross-disciplinary study allows in-depth investigations of native knee deformity and postoperative realignment. Data on larger numbers of patients are needed to provide statistical evidence, but these preliminary results demonstrate slightly better efficacy of knee osteotomy combined with meniscal allograft transplantation, which is currently associated with similar inflammatory values between the two patient groups.

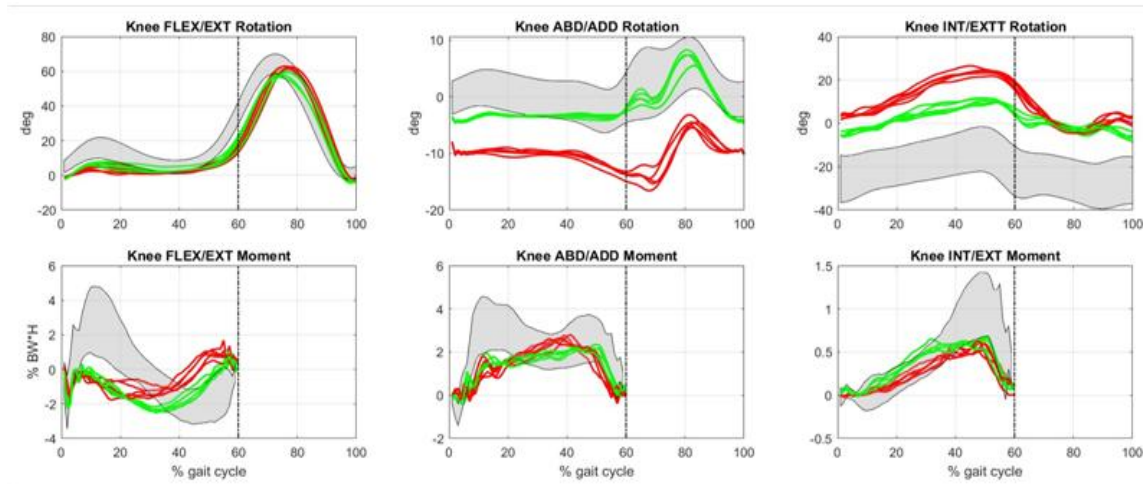


Figure – Pre-operative (red curves) and post-operative (green curves) kinematic (upper figures) and kinetic (lower figures) patterns on the three anatomical planes. Data from a representative patient.

Po4_2.10

Dxa-based 3d finite element models predict hip fractures better than bmd in ostpre cohort

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Abstract

Introduction. Bone strength is a major contributor to the bone's susceptibility to fracture. Fracture risk is estimated by combining epidemiological risk factors with dual-energy X-ray absorptiometry (DXA) derived areal bone mineral density (aBMD). However, aBMD is a moderate predictor of bone strength¹, which is better captured by 3D finite element (FE) models. The clinical use of FE models is hindered by need for CT images and lack of automation. Previously, we developed an automatic method that reconstructs 3D shape and internal density distribution of the hip based on a single 2D DXA image, followed by a subject-specific FE model to predict femoral strength². We now evaluate the method's ability to predict hip fractures in a population-based cohort of women.

Methods. OSTPRE cohort includes 3222 females aged 47-56 years at baseline, for which DXA scans were taken at 5-year intervals³. We performed a study with cases and controls with 46 women with a hip fracture occurring within 10 years from a DXA scan and with no previous osteoporotic fractures, and 2 healthy control cases to each hip fracture case, matched by age, height and BMI (table 1). Subject-specific 3D FE models of the proximal femur were automatically reconstructed from the 2D DXA scans. Femoral strength was predicted for 10 different configurations of a fall to the side using a validated strain-based criterion⁴. Logistic regression analysis with 10-fold cross validation was performed to compare the fracture predictive ability of aBMD+BMI to that of FE-predicted femoral strength, using the area under the receiver operating characteristic curve (AUROC).

Results. The AUROC was significantly higher (p-value=0.016, using Delong method) for the FE-predicted femoral strength (AUROC=0.74) when compared to aBMD+BMI (AUROC=0.64).

Discussion. We used 3D FE models obtained from a 2D DXA scan to predict incident hip fractures in OSTPRE cohort. Our results indicate that FE models performed better than aBMD+BMI. This is the first time 3D FE models reconstructed from 2D DXA scans have outperformed aBMD in a prospectively-followed female cohort. These results complement our previous findings on a cohort of elderly men⁵ to prove the generality of our approach. The method is automatic and has the potential to improve the accuracy of fracture risk predictions without additional costs compared to the current clinical practice.

References. 1.Stone et al. (2003). J.BoneMiner.Res.; 2.Väänänen et al. (2015). MedImageAnal; 3.Sund et al. (2014). Calcif.TissueInt.; 4.Schileo et al. (2014). J.Biomech.; 5.Grassi et al. (2023). J.BoneMiner.Res.

	Hip fracture cases and controls (N=138)	
	Hip fractures (N=46)	Controls (N=92)
Age (years)	75.0 ± 2.8	74.9 ± 2.8
Height (cm)	158.8 ± 5.4	158.7 ± 5.4
Weight (kg)	71.1 ± 13.8	70.3 ± 12.8
BMI (kg/m²)	28.2 ± 5.3	27.9 ± 4.8
Total hip <u>aBMD</u> (g/cm²)	0.75 ± 0.10	0.83 ± 0.14
Mean FE-predicted strength (BW)	5.1 ± 0.2	5.9 ± 0.4

Table 1: Mean and standard deviation characteristics for the sub-cohort analyzed in this study.

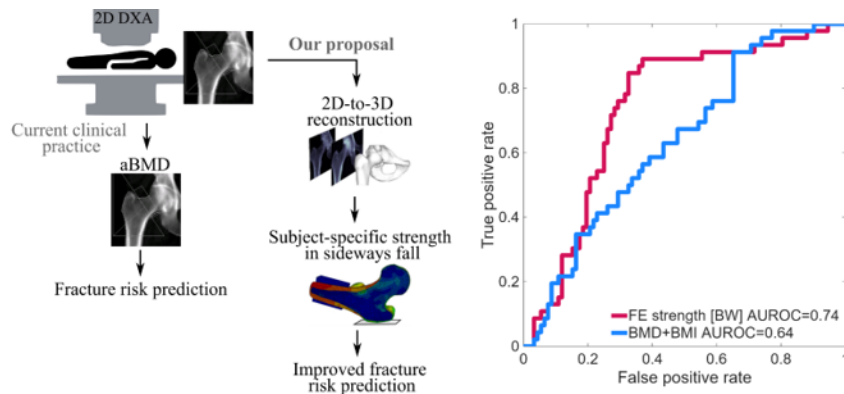


Figure 1: Left, schematic of our proposal compared to the current clinical practice. Right, ROC curve for FE-predicted femoral strength and aBMD+BMI when comparing hip fractures and healthy controls in OSTPRE cohort.

Po4_2.11

Understanding the crosstalk between mechanical stimulation, macrophage polarisation and MSC differentiation: An in vitro model to simulate early events during bone fracture healing

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Abstract

Introduction. Bone healing is a complex scenario with a plethora of mechanisms working in concert. In this context, the immune system, particularly the effect of macrophages on mesenchymal stromal cell (MSC) recruitment and differentiation, cannot be overestimated. However, evaluations with cells cultured in 2D and under static condition fail to recapitulate this. By use of an in-house designed bioreactor to culture cell-scaffold constructs under compression and shear, we move towards in vitro models with higher predictability. We aim to gain in-depth understanding of the crosstalk between mechanical stimulation, immune system, and endochondral ossification.

Material and Methods. THP-1 were seeded in fibrin hydrogels and cultured in standard expansion medium (M0) or medium supplemented with LPS and IFN- γ , or IL-4 to induce an M1 or M2-like phenotype, respectively (named M(LPS) or M(IL-4)). Cell-fibrin constructs were cultured under static conditions, or in a bioreactor. 20% compression load and shear at a frequency of 1 Hz were applied for 1 hour per day over 3 days. Protein or gene expression levels were analysed with ELISA or qRT-PCR, respectively. Conditioned media was collected and added to MSC pellets. Gene expression and glycosaminoglycan (GAG) deposition were analysed after 9 days in culture.

Results. Gene expression levels in THP-1 cells differed in response to culture in 3D vs. 2D and under mechanical load vs. static culture. In 2D, phenotypes could be divided into classically or alternatively activated macrophages with clear upregulation of pro- or anti-inflammatory genes, respectively. In 3D fibrin hydrogels, boundaries between different phenotypes were indistinct. Mechanical stimulation induced changes of both pro- or anti-inflammatory hallmarks. In M(IL-4) conditions, expression of IL-1 β , IL-6, TNF- α , IL-10, CCL18, CD163, and CD206 was increased compared to the static condition. In M(LPS) conditions, a less pronounced up or down-regulation in response to mechanical load was observed. After treatment with conditioned media, the majority of assessed genes in MSCs remained unchanged, with few exceptions: while IL-8 decreased, ADAMTS4, RUNX2, ALPL,

and COL2 increased in all conditions treated with medium from mechanically stimulated THP-1 cells.

Discussion and Conclusion. Our results suggest that culture in 3D and under mechanical loading induces a complex macrophage polarisation spectrum, different to observations in 2D. MSCs cultured with conditioned media responded in a donor-dependent manner, underpinning clinical observations of patient-specific symptoms to local inflammation. Ultimately, we aim to be able to suggest an appropriate loading or resting scheme for patients with bone fractures.

Po4_2.15

Unraveling the Mechanics of Buckyball Microscaffolds: A Computational Approach

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Abstract

Tissue engineering and regenerative medicine is rapidly advancing, with scaffolded spheroids emerging as critical building blocks for regenerating damaged tissues. These three-dimensional structures aim to improve cell retention, reduce compaction, and enhance biocompatibility. To achieve these goals, the mechanical properties of the microscaffolds are of paramount importance.

This study focuses on assessing the elastic behavior of a specific type of microsccaffold: truncated icosahedron-shaped structures, commonly known as "buckyballs." Fabricated by means of 3D printing, these structures are designed for application in the nucleus pulposus of intervertebral discs. Specifically, the buckyballs under investigation are made of a PCL-based resin, with cross-linkable gelatins also considered for comparison. We present numerical modeling approaches to predict the elastic deformation characteristics and key elastic constants (such as Young's modulus) of both individual buckyballs and buckyball assemblies.

For individual buckyballs (exhibiting sizes of 200 and 300 microns), we developed a Finite Element model using the commercial software Abaqus and subjected the model to uniaxial compression. Comparing the computationally obtained load-displacement curves with experimental results validated this approach. Furthermore, we investigated various cross-sectional shapes of the individual struts to study the potential effects of unintentional 3D printing artifacts (i.e., deviations from a circular cross-section). To study the mechanical behavior of buckyball assemblies, as they would be employed in intervertebral discs, we employed a Finite Element analysis-based periodic homogenization approach. This involved analyzing a representative unit cell subjected to six specific strain states. The resulting buckyball deformations were then used to determine the corresponding stiffness tensor components of the overall assembly.

In summary, our computational buckyball models elucidate the effects of specific, technologically relevant characteristics on the elastic behavior of individual buckyballs and buckyball assemblies. This establishes computational modeling as a valuable complement to potentially laborious experimental studies, ultimately facilitating the clinical application of such microscaffolds.

Po4_2.17

Superior-Segment Bilateral Facet Violation in Lumbar Transpedicular Fixation: A Biomechanical Study

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Abstract

Objective. The facet joint is a crucial dynamic stabilizer of the lumbar spine, and facet joint violation (FJV) caused by pedicle screws is a common complication in lumbar fusion. However, the impact of this complication on lumbar spine kinematics remains unclear. This study aimed to investigate the biomechanical changes in lumbar spine motor units following bilateral FJV in cadaveric specimens and analyze these variations under different loading conditions.

Methods. Range of motion (ROM) testing was performed on 24 human cadaveric lumbar spines (L4-S1) under flexion-extension, lateral bending, and axial rotation loads based on inclusion and exclusion criteria. The specimens were randomly assigned to four groups based on the degree of FJV. G0 (Control Group): No violation; G 1 (Screw Head Blocking Group): Screw heads block the facet joints; G2 (Mild Violation Group): Partial screw penetration of the facet joints; G3 (Severe Violation Group): Complete screw penetration of the facet joints. Each group included six specimens. After initial testing, pedicle screws were inserted into the L5 vertebra, and biomechanical testing was repeated. Full ROM at the proximal adjacent levels under different loading moments (7.5, 6.0, 4.5 Nm) was recorded and normalized to the intact state (100%). Relative changes were compared among the four groups.

Results. In the control group (G0), supradjacent-level ROM showed no significant differences compared to the intact, noninstrumented spine under any loading moment. G1: Supradjacent-level flexion-extension ROM significantly decreased under all loading moments (7.5, 6.0, 4.5 Nm) compared to the intact spine. Additionally, lateral bending ROM significantly decreased at 4.5 Nm ($P < 0.05$). G2: Supradjacent-level ROM for lateral bending and axial rotation significantly increased under all loading moments compared to the intact spine ($P < 0.05$). G3: Supradjacent-level ROM significantly decreased under all loading moments ($P < 0.05$), except for lateral bending ROM at 7.5 and 6.0 Nm.

Conclusion The biomechanical effects of bilateral facet violation depend on its severity. Screw Head Blocking: This may cause a reduction in ROM, particularly in flexion-extension and lateral bending, potentially leading to persistent low back pain due to frequent impingement. Mild Violation: Increased ROM in lateral bending and axial rotation may predispose to lumbar instability and adjacent segment disease. Severe Violation: Reduced ROM in flexion-extension and axial rotation, along with changes in lateral bending, may accelerate degeneration of the supradjacent segment. Careful attention to pedicle screw placement is essential to minimize the risk of bilateral FJV and its associated biomechanical complications.

Po4_2.19

Impact of Robotic-Assisted Unicompartmental Knee Arthroplasty on Tibial Periprosthetic Fracture: A Comparative Analysis to Conventional Approach

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Abstract

Introduction. Recent advancements in robotic-assisted surgery have significantly enhanced the precision of implant positioning and the meticulous balancing of soft tissues during surgical procedures. Despite these innovations, the relationship between robotic assistance and the risk of periprosthetic tibial fractures in unicompartment knee arthroplasty (UKA) remains ambiguous and requires further investigation. This study compares the incidence of periprosthetic tibial fractures to conventional UKA methods when utilizing robotic-assisted burring techniques. By examining the outcomes of both approaches, we aim to provide more precise insights into the safety and effectiveness of robotic assistance in knee arthroplasty procedures.

Methods. Twelve fresh-frozen full lower-extremity cadaveric specimens were divided equally into two groups: conventional cemented UKA and robotic-assisted burring (CORI, Smith & Nephew). Both utilized the Journey II UNI implant. After surgery, the tibias were isolated and subjected to axial loading using a servo-hydraulic material testing machine (MTS) until macroscopic fractures occurred. Fracture patterns were evaluated via macroscopic examination and fluoroscopy (Figure 1). Results were normalized to body weight (BW%) to account for variability in age and BMI.

Results. The robotic group demonstrated a higher mean load to failure of 4394.83 N (SD = 1198.20), compared to 3559.33 N (SD = 1861.81) in the conventional group, translating to a 23.37% greater resistance to fracture. Table 1 shows the raw data from each sample. However, the difference was not statistically significant ($p = 0.3760$). Fractures in the robotic group were localized to the metaphyseal region, with articular depression as the primary pattern. In contrast, conventional UKA fractures typically propagated from the sagittal cut to the proximal diaphysis, suggesting less precise cutting as a contributing factor.

Conclusion. While the difference in fracture resistance observed in robotic-assisted unicompartmental knee arthroplasty (UKA) was not statistically significant, the study revealed noteworthy biomechanical advantages. Specifically, robotic-assisted UKA exhibited enhanced fracture resistance and demonstrated more localized fracture patterns compared to traditional methods. These results underscore the promising potential of robotic-assisted techniques to improve surgical accuracy and lower the likelihood of

fractures during the procedure. To validate these initial findings, further research with larger sample sizes and various implant systems will be essential.

Figure 1: Experimental setup demonstrating the specimen secured in the MTS with axial loads applied (indicated by the blue arrow) until a periprosthetic fracture.



Table 1 displays load to failure [N] in force and as a percentage of body weight (BW).

	Specimen No	Age (Yrs)	Sex	BMI	BW (lbs)	BW (Kg)	Side	Load To Failure [N]	%BW (lbs)	%BW (Kg)
Conventional	1	87	F	24.03	140	63.50288	L	824	588.571429	1297.57894
	2	81	M	25.54	168	76.203456	R	5209	3100.59524	6835.64798
	3	92	F	35.15	180	81.64656	R	2629	1460.55556	3219.97644
	4	85	M	25.68	200	90.7184	R	2633	1316.5	2902.38805
	5	69	M	23.99	172	78.017824	L	5764	3351.16279	7388.05532
	6	81	F	33.28	200	90.7184	R	4297	2148.5	4736.63557
	Avg	82.5		27.945	176.666667	80.1345867		3559.33333	1994.31417	4396.71372
	SD	7.79102047		4.94485086	22.5447703	10.2261274		1861.81467	1077.87868	2376.31765
Robotic	1	86	F	19.93	109	49.441528	L	3185	2922.01835	6441.95301
	2	64	M	23.15	166	75.296272	L	5356	3226.50602	7113.23397
	3	67	M	22.27	138	62.595696	R	4157	3012.31884	6641.03168
	4	93	M	19.52	140	63.50288	L	6219	4442.14286	9793.25662
	5	83	F	19.02	104	47.173568	R	3220	3096.15385	6825.85638
	6	68	F	36.27	218	98.883056	R	4232	1941.2844	4279.803
	Avg	76.8333333		23.36	145.833333	66.1488333		4394.83333	3106.73739	6849.18911
	SD	12.0235879		6.53064162	41.9924596	19.0474438		1198.20156	799.88511	1763.44625
Conventional VS Robotic	p=0.3555			p=0.3368	p=0.2568	p=0.2568		p=0.3760	p=0.8415	p=0.325

Po4_2.21

How accurately can surgeons perform angle manipulation? Quantitative assessment of the accuracy of manual angle manipulation of orthopedic surgery: a cadaver study

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Abstract

Introduction: To date, only few studies have been performed on the accuracy of manual angle manipulation during orthopedic surgery. This cadaver study was aimed at quantitatively assessing the accuracy of manual angle manipulation performed by orthopedic surgeons according to their surgical experience and comparing it with manipulation performed with the assistance of a digital goniometer.

Material and methods: Six lower-leg specimens of fresh-frozen human cadavers were subjected to angle manipulation performed via Kirschner wire (K-wire) insertion. K-wires were inserted manually and with the assistance of a digital goniometer at target angles of 0°, 30°, and 60° by three operators who had different levels of experience in orthopedic surgery. The accuracy of the insertion angles at the target angles was evaluated using computed tomography.

Results: The mean angle error in the manual angle manipulation was 8.8° (standard deviation [SD], 6.0). When the target angles were set to 0°, 30°, and 60°, the identified angle errors were 6.1° (SD, 4.3), 8.8° (SD, 6.6), and 11.7° (SD, 5.6), respectively, and each value did not show any significant difference among the operators. With the assistance of a digital goniometer, the mean (SD) angle error was significantly improved to 2.1° (1.1°) ($p < 0.001$). The amount of improvement in accuracy significantly increased as the target angle increased ($p = 0.01$).

Conclusion: This cadaver study quantified the inaccuracy of manual angle manipulation in orthopedic surgery and showed that these inaccuracies can be improved using an assistive device. These results support the need to develop a device that can compensate manual angle manipulation in orthopedic surgery.

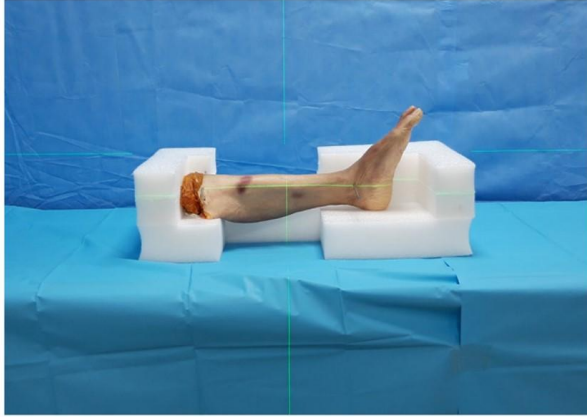


Fig. 1 Two Steinmann pins are inserted on the proximal and distal parts of the lower leg and laid parallel to the operating table on the custom-made holder. The position is confirmed with the use of a laser



Fig. 2 K-wires are inserted for the target angle on the tibia from the medial side of the specimen. The target angle is randomly selected as 0°, 30°, or 60°, and insertions are performed manually and with assistance of the digital goniometer

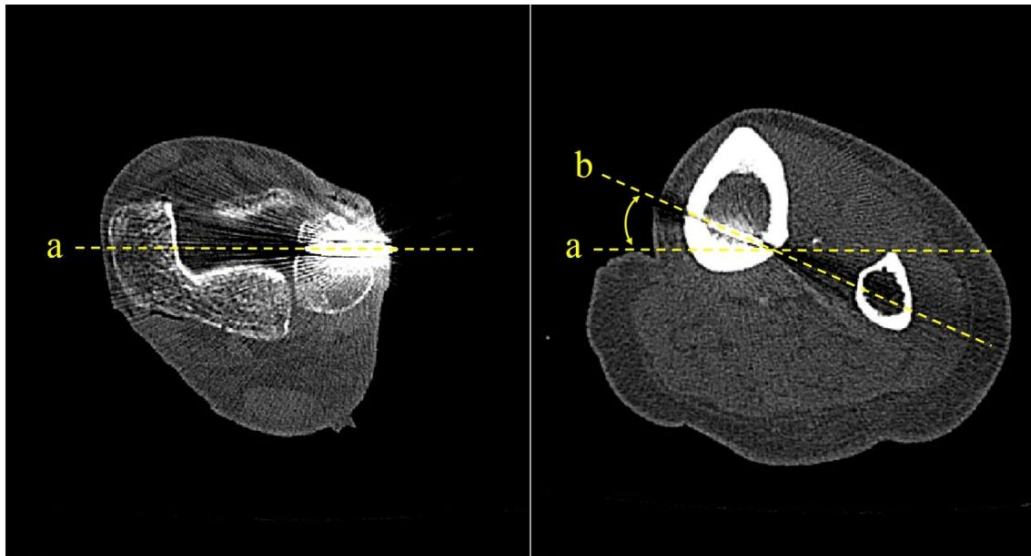


Fig. 5 Insertion angle is the angle between the Steinmann pin (a) and the inserted K-wire (b) measured on computed tomography. The difference between the insertion angle and target angle is regarded as angle error

Po4_2.22

Piezo1 Conjugated Nanoparticles Induced Osteogenic and Anti-osteoclastogenesis Activation Regulated by Dynamic Magnetic Stimulation

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Abstract

Current osteoporosis treatments target either osteoclast or osteoblast activity, specifically, and very few can simultaneously regulate both osteogenic and anti-resorption activities. Magnetic actuation of the mechanosensitive ion channel Piezo1 (P1) using superparamagnetic iron oxide-gold (SPIO-Au) core-shell nanoparticles (NPs) is a novel approach to enhancing the activity of bone cells. Hence, this study hypothesizes that the dynamic magnetic field (DMF) stimulated P1-SPIO-Au NPs can promote both osteogenesis and inhibit osteoclastogenesis.

SPIO-Au NPs were synthesized and P1 antibody was attached to the NPs using carbodiimide chemistry.² DMF was created by rotating a Halbach array of magnets (0.5T, 0.5Hz) around a 35-mm dish. 4 groups were evaluated: (1) Control, (2) NPs, (3) DMF, (4) DMF+NPs. Ca²⁺ influx in MC3T3-E1 pre-osteoblast cells was measured for 3 min after 30 min of DMF via confocal microscopy. MC3T3-E1 cells were differentiated, underwent DMF treatment for 30min/day for 7 or 14 days, and then stained for ALP. Pre-osteoclast RAW 264.7 cells were differentiated, subjected to DMF treatment for 30min/day for 5 days, and stained for TRAP activity. TRAP secretion was analyzed using ELISA after 0, 3, and 5 days.

DMF-stimulated NPs significantly increased Ca²⁺ influx compared to DMF, NPs, and control (p<0.05, p<0.01, and p<0.001, respectively). DMF-stimulated NPs significantly increased ALP staining compared to NPs and control at day 7 (p<0.05) and at day 14 (p<0.0001). There was no significant difference between DMF and DMF-stimulated P1-SPIO-Au NPs at day 7 but was significant at day 14 (p<0.0001). NPs, DMF, and DMF-stimulated NPs significantly reduced the formation of osteoclasts compared to the control (p<0.001, p<0.05, p<0.0001, respectively). DMF-stimulated NPs resulted in the most significant inhibition of osteoclast formation, but this reduction was not significant compared to that of NPs. TRAP secretion had a similar trend as the TRAP staining results at Day 3 and was more significant at Day 7.

The ability to magnetically activate P1 functionalized SPIO-Au NPs has been demonstrated to enhance osteoblasts' mechanosensitivity. It is indicated that DMF stimulation of NPs significantly increases the Ca²⁺ influx and ALP activity in osteoblast cells, which may further regulate proliferation and differentiation. Interestingly, it has been shown that DMF stimulation of NPs resulted in reduced osteoclast formation as well. In summary, this study suggested the potential for a novel bone regeneration approach by simultaneously activating osteoblasts and inhibiting osteoclasts, which may pave the way for future osteoporosis treatments.

Po4_2.23

Investigation of the influence of screw position and screw insertion on the local micro-mechanics of the fracture gap and the interfragmentary movement

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Abstract

Background: Femoral fractures are common injuries in orthopaedic trauma surgery that usually require surgical intervention with internal fixation devices such as intramedullary nails or implants. In the implant case studied here, the number of screws, their positioning and the corresponding angle of insertion can significantly influence the mechanical environment at the fracture site, which in turn can affect healing outcomes. A better understanding of how screw configurations affect local micromechanics and interfragmentary motion can be used to optimize patient-specific implant setups.

Methods: 3D computer models were generated based on clinical image data of several femurs from body donations. These models were virtually provided with different fractures that would be treated with implants in everyday clinical practice and then virtually treated accordingly. To investigate the influence of the screw configuration, a series of configurations was then created for each model and analyzed using biomechanical FEM. To ensure realistic boundary conditions, physiological loading conditions were applied as hip forces and moments corresponding to those of normal healing progression from partial to full weight bearing. The key parameters evaluated included the von Mises stress distribution of the implant and the associated screws as well as the degree of interfragmentary movement and the strain-state of the fracture gap.

Results: The simulations showed that there are combinations of fractures and screw configurations where the influence on the micro-mechanics is greater than in other cases where the screw variation exerts only a minor influence. In addition, the result is influenced by the quality of the bone, comparing healthy bone versus osteoporosis patients.

Conclusion: The study shows that there is an influence of the screw configuration, which can be of great relevance in individual cases. The mechanical stability of the bone-implant system also influences the interfragmentary movement depending on the level of the partial weight bearing.

Po4_2.24

Electromyography and ultrasound scan analysis of muscle activity during virtual reality dynamic plank exercise on icaros pro machine.

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Abstract

Objective: We aim to study muscle activity during simulated virtual reality space flights in dynamic plank positions.

Method: Sixteen (16) subjects age range (21-55yrs old) underwent ultrasound scan (Clarius) of the rotator cuff muscles and upper limb arterial blood flow dynamics to exclude rotator cuff arthropathy and occlusive arterial disease.

Four activated electromyography (EMG) sensors (Delsys Europe) were then installed on each subject. Four EMG sensors were applied per flight. On the first flight, one sensor was applied to each right and left trapezius and erector spinae muscles. Following full recovery seven days later, second flights were conducted with sensors applied to the right and left trapezius and erector spinae muscles. All subjects were sequentially mounted on an icaros pro machine for a simulated virtual reality space flight on "Eden track" with PICO 4 headset. The icaros pro machine (icaros Germany) braces joints and allows for muscle, tendon, bone and ligament stimulation because of the subject assumed dynamic pre and in-flight positions. "Eden track" is a virtual reality icaros track. Described as an earth like planet captured by humanity year ago. Its thick atmosphere allows for speeds of 216 km/h to 324 km/h. Attentive cerebral and truncal control are essential in-flight to overcome the high torque force-moments that must be generated for a fast flight completion. A predominant cerebral neuro active attentive flight simulated through virtual reality headset (PICO 4) then occurred.

Results: Ultrasound scan of all subjects confirmed no rotator cuff arthropathy or occlusive arterial disease. Bloodflow characteristics of the brachial and radial arteries were normally biphasic in all subjects.

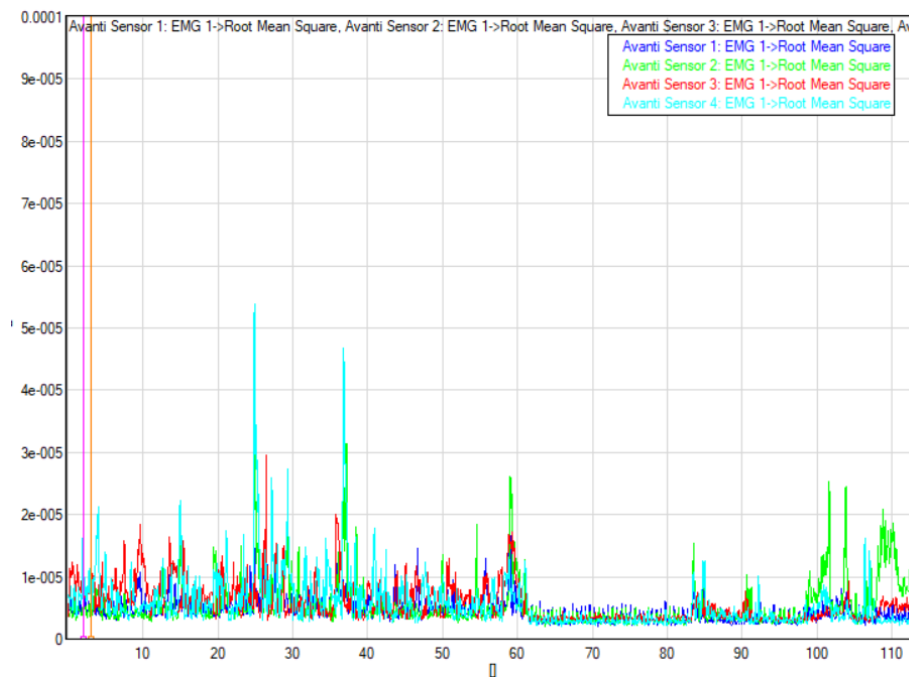
Time dependent Torques:

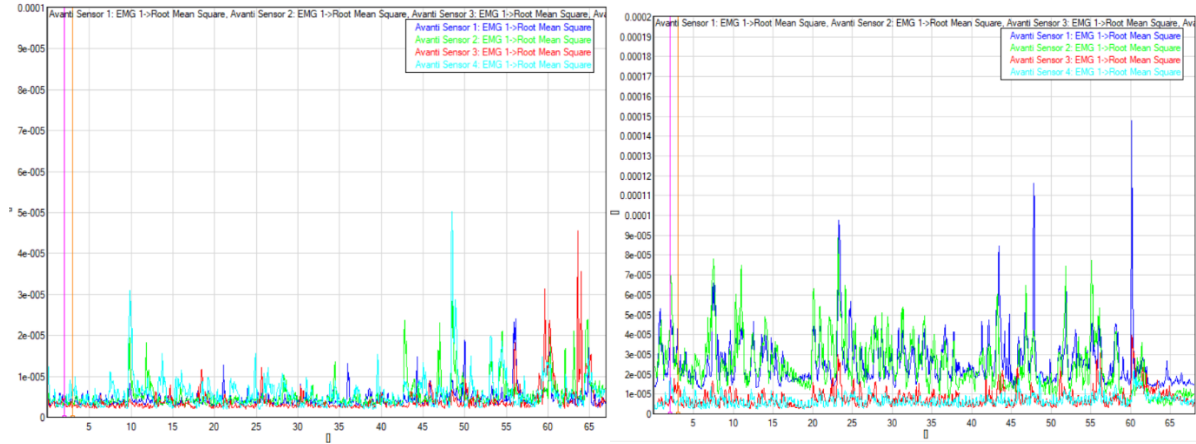
The best Eden flight times range of 70-100s were achieved.

Two major torque moments occur on Eden track. The first occurs at approximately 20s into flight. This is a predominant concave to the left torque with a duration of 5 secs. The second torque moment occurs in the later half of the simulation within 15 seconds of the end of the simulation. This is a predominant concave to the right torque. Both torques create statistically significant increased EMG muscle activity.

EMG results

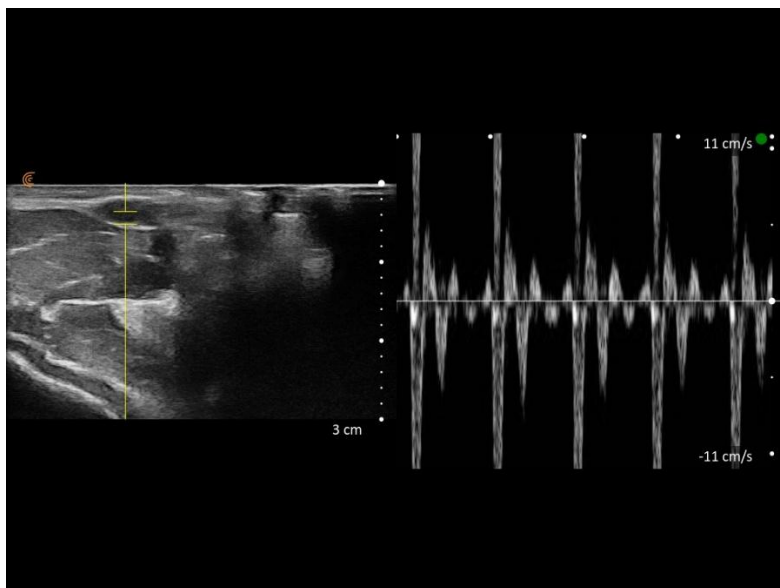
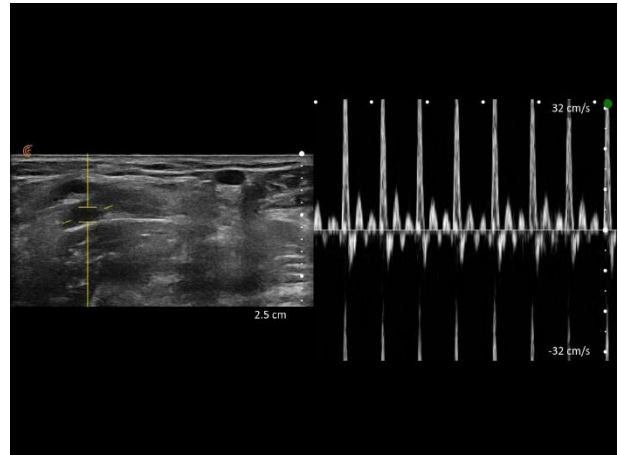
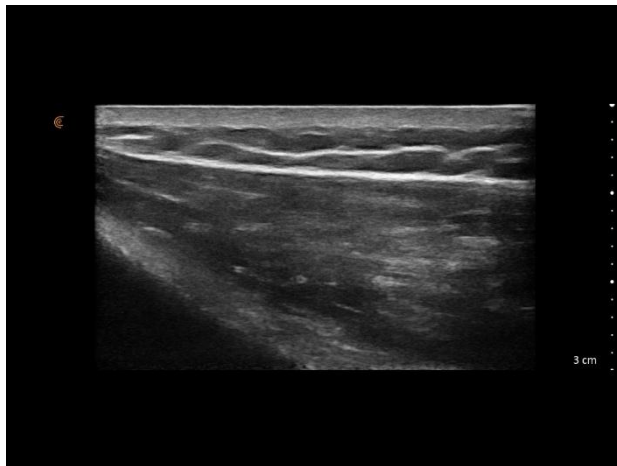
The root mean squared valued of all EMG sensors data were calculated and plotted to evaluate statistically significant muscle activity and differences between upper and lower spinal segments. All subject exhibited higher emg signals in the upper trunk trapezius muscle sensors, than the signals in the lower trunk erector spinae muscles sensors. The torque topography of the icaros pro “Eden track” was also reflected on the time dependent sensor data. This is depicted as the left sided sensors reflected higher muscle activity during a left sided concave torque and the right sided sensors reflected higher muscle sensors activity during a right sided concave torque.





Ultrasound scan results

All subjects exhibited healthy rotator cuff tendon with good biphasic blood flow dynamics in both brachial and radial arterial systems.



Discussion:

Utilising the dynamic plank position with a virtual reality head set on the icaros pro machine creates a position of optimum advantage for the neuro cerebral complex. The head and vertebral spine are in extension. Johnson et al 2012 previously demonstrated that the head extended position increases sensory motor integration, somatosensory evoked potential and spinal cord velocities. In our study visual sensory, vestibular, and somatosensory systems provide the central nervous system with an updated body schema of the biomechanical and spatial position of the body whilst making decisions at an average speed of 270 km per hour. Flight completion, the desired outcome in this study, is therefore a highly complex task, requiring higher order integration from joint proprioceptors, muscle mechanoreceptors, with vestibular and visual feedback to control head position, head orientation, and whole body posture. The engineering design of the icaros pro creates a protective mechanism for lower lumbar spinal segments loads, as the erector spinae EMG muscle signals are consistently and statistically significantly lower than the EMG signals of the upper trunk, trapezius muscles. This is to our knowledge the very first ever scientific study of the effect of virtual reality icaros pro positively impacting lower lumbar spinal segment in healthy subjects with ultrasound confirmed intact rotator cuff muscles and blood flow mechanism.

Conclusion: Virtual reality icaros pro flight simulated in a dynamic plank position stimulate muscle activity efficiently and protects the lower spinal segments. The efficacy of muscle activity in a joint protective neuro-active environ offers an attractive alternative to traditional rehabilitative methods. The potential impact for utilising the icaros pro for sports training, injury prevention and rehabilitation, as well as for post surgical treatments are enormous.

Po4_2.25

Medial Opening-Wedge High Tibial Osteotomy Alters Three-Dimensional Knee Joint Biomechanics and Trunk Posture

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Abstract

Introduction: Knee osteoarthritis (KOA) is a common degenerative condition that leads to pain, disability, and reduced quality of life. Medial open-wedge high tibial osteotomy (MOWHTO) is a surgical procedure designed to correct alignment and alleviate symptoms by redistributing load. While previous studies have focused on the coronal plane, the impact of MOWHTO on sagittal plane biomechanics and trunk posture remains unclear. This study aimed to evaluate three-dimensional biomechanical changes in the knee joint and trunk posture following MOWHTO. We hypothesized that MOWHTO would result in significant biomechanical changes not only in the coronal plane but also in the sagittal plane, with improvements in trunk posture.

Material and Methods: This retrospective study included 55 patients (55 knees) with medial compartment KOA (Kellgren-Lawrence grade ≥ 2) who underwent MOWHTO. Pre- and postoperative gait analyses were conducted using a three-dimensional motion capture system and force plates to assess knee joint moments, trunk posture, and radiographic parameters. The MOWHTO procedure involved medial wedge correction targeting a 62.5% mechanical axis shift using TomoFix or TriS plate systems. Knee joint moments (adduction moment [KAM], flexion moment [KFM], and rotation moment [KRM]) including total joint moment (TJM), and radiographic parameters (hip-knee-ankle angle [HKA], percentage of mechanical axis [%MA], and femorotibial angles [FTA]) were evaluated. Clinical outcomes were assessed using the Japanese Knee Osteoarthritis Measure (JKOM) and Knee Society Score (KSS). Paired t-test was performed for statistical analysis between pre and postoperative results. Statistical differences were considered significant for values of $p < 0.05$.

Results: Postoperatively, significant reductions in KAM ($P < 0.01$) and TJM ($P < 0.001$) were observed, with a shift in the proportional contribution from %KAM to %KFM. Knee flexion moments (KFM) remained unchanged pre- and postoperatively. Knee varus angles decreased ($P < 0.01$), and flexion angles improved during gait ($P < 0.05$). Trunk flexion angles significantly decreased, indicating better postural alignment ($P < 0.01$). Radiographic analysis demonstrated increased HKA angles and %MA, along with reduced FTA ($P < 0.01$). Clinical outcomes showed significant pain relief and functional improvement ($P < 0.01$).

Discussion: MOWHTO not only reduces medial knee loading but also significantly improves the gait in three-dimensional moment changes, and impacts sagittal plane biomechanics and trunk posture. These changes suggest enhanced gait efficiency and reduced compensatory strategies postoperatively. The results underscore the need to consider both coronal and sagittal biomechanical effects in evaluating MOWHTO outcomes, highlighting the comprehensive benefits of this intervention for medial compartment KOA.

Po4_2.26

Enhancing Protein Interaction Networks in IVDD Using Machine Learning Link Prediction

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Abstract

INTRODUCTION: Intervertebral disc degeneration (IDD) involves imbalanced anabolic and catabolic regulation of the disc extracellular matrix. Biological regulatory network models (RNM) are insightful, though protein-protein (p-p) interaction topologies are challenging to adjust. A previously developed RNM predicted baseline non-degenerate and degenerate states and rescue strategies for nucleus pulposus cells (NPC). Yet, some functional wiring upon IL-4 stimulations remained unclear due to limited data. Accordingly, we propose a machine learning-based link prediction to test and discover p-p for NPC RNM.

METHODS: A link prediction model was developed to uncover missing p-p interactions, starting from a validated NPC RNM representative of non-degenerate discs (Fig.1A). It integrated: (i) p-p interactions from proteomics of human NPC stimulated by IL-1 β or IL-4; (ii) a protein-Gene Ontology (GO) term association network; (iii) a GO term relationship network for functional connections. Network connectivities were split into training and testing datasets, respectively with existing and non-existing links. Using Node2vec, we generated node embeddings, concatenated into feature vectors, and labeled for link prediction. Optimal hyperparameters, (ROC-AUC ≥ 0.80) were identified to train a Support Vector Machine (SVM) classifier and predict new potential p-p interactions. Probabilistic scores evaluated the strength and reliability of the predictions.

RESULTS: The final model demonstrated robust performance (ROC-AUC = 0.868), indicating high capacity in distinguishing existing from non-existing interactions. The network consisted of 4,072 nodes and 6,557 links, with community detection performed using the Louvain method (Fig.1B). Optimal hyperparameter configuration was found (dimension=154, walk length=20, number of walks=100, epochs=20), leading to 58 new p-p

interactions, each with a probability higher than 0.7 (Fig.1C). The initial validity of the NPC RNM simulations remained unaltered.

DISCUSSION: The link prediction model demonstrated a very good predictive performance for p-p interaction predictions. While new links did not alter the simulation results by the initial NPC RNM, showing the robustness thereof, they revealed interactions still unreported for the IVD but supported by mechanistic analyses of the regulation pathways: IL-6 and IL-8 inhibition by TGF- β via the SMAD pathway, and IL-1 β and TNF inhibition by IGF via the PI3K/Akt pathway. The present p-p interaction prediction model appears promising to identify new links in biological networks, potentially important to inform experimental and clinical IDD research.

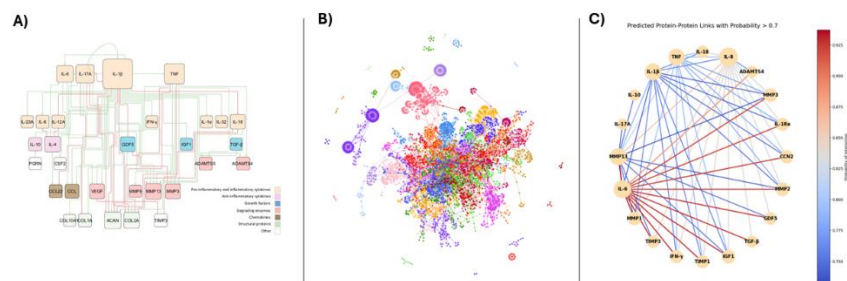


Figure 1: Link prediction model network. An initial p-p network (A) was merged with two other networks including GO terms (B). The model was trained on the merged network and predicted new links (C).

Po4_2.27

Preclinical characterization of Mg-Zn-Ca implants biomechanics, by using ovine tibia model

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Abstract

INTRODUCTION: Implant removals due to complications with non-biodegradable orthopedic implants place a significant burden on our healthcare system and the well-being of patients. Consequently, scientific research has increasingly focused on exploring bioresorbable materials for implant manufacturing.

Methods: In this study, bone screws made of a magnesium alloy (ZX00 Mg-0.45 wt% Zn-0.45 wt% Ca), a polymer (PLGA), and titanium (Ti) were tested using pull-out and push-out methods in an ovine tibia model. The fixation strength of the implants was tested at 0, 3, 6, and 12 weeks. Monocortically implanted screws were tested by pull-out, while bicortically implanted screws were tested by push-out tests. Additionally, ex vivo μ CT imaging was performed to measure the contact lengths between the implant and bone as seen in figure 1. The fixation forces were normalized to a single thread revolution after measuring the contact length. Statistical analysis was performed using 2-way ANOVA.

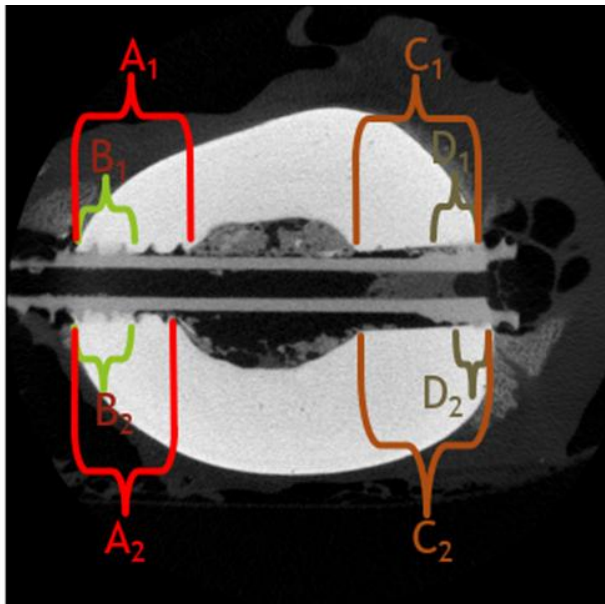


Figure 1: Transverse μ CT cross section view of a bicortically implanted 3.5x24mm ZX00 compression screw in a sheep tibia. Brackets mark the areas where diaphyseal thickness(A&C) and contact length(B&D) were measured.

Results: The results of the ZX00 alloy in both experimental methods showed no statistically significant differences in fixation strength between the time points of 0, 3, 6, and 12 weeks. Thus, the bioresorbable ZX00 alloy exhibited constant fixation strength throughout the entire experimental period. These findings were reinforced as the forces for the ZX00 alloy at different time points also showed no significant differences after normalization (Fig. 2). Furthermore, a reduction in the relative standard deviation was observed after normalization of the forces.

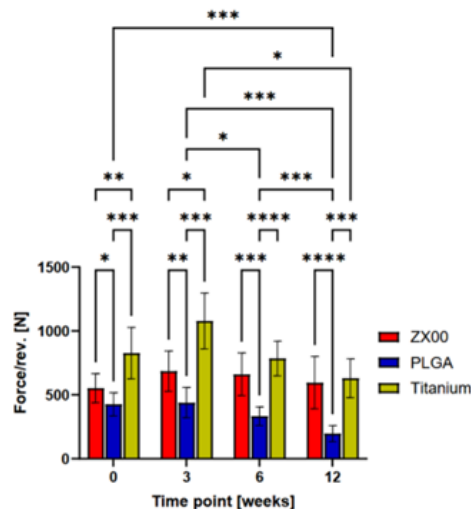


Figure 2: **Error bar plot of push-out test results normalized to a single thread revolution**

Push-out tests were performed on cannulated bicortical screws ($d = 3.5$ mm, $l = 24$ mm). ZX00, PLGA and Ti were tested at time points 0, 3, 6 and 12 weeks. SD is shown by whiskers. A two-way ANOVA was performed for statistical interpretation. Statistical significance is indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Conclusion: The bioresorbable properties of ZX00, coupled with its ability to maintain fixation force and its composition of only endogenous materials, lead to the conclusion that ZX00 is not only a safe but also a healthy implant material.

Po4_2.28

Impact of Computational Models on the Outcome of FE Biomechanical Simulations

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Abstract

Biomechanical finite element simulations are becoming increasingly popular and essential for evaluating healing potential and making early predictions in clinical and research settings. A key factor influencing the accuracy of these simulations is the origin and quality of the data.

It is already known that the segmentation of the data has an influence on the simulation [1]. But what about the origin of the data for the computational model?

This study investigates the impact of three distinct implant data types on simulation outcomes: segmented implant data derived from a computed tomography scan, reverse-engineered implant data obtained through 3D scanning of an implant, and the original dataset provided by the manufacturer. The goal is to determine how the choice of data type affects simulation accuracy.

Segmented implants eliminate the need for alignment within the bone model but may lose critical features, such as screw threads, due to smoothing processes. Reverse-engineered implants offer greater detail but are influenced by the quality of the 3D scanner and the reconstructed surface, with noise and scan artefacts potentially compromising precision. Manufacturer-provided datasets retain the original geometry but require manual alignment with the bone model, introducing additional variables that affect the simulation's outcome.

The study is based on a segmented dataset of a radius with an artificially generated fracture (AO Classification Type 2R1A2) derived from a computed tomography scan. Each implant type was integrated into the bone model, and clinically relevant load scenarios were simulated. Results were evaluated based on mechanical parameters such as deformation, strain and stress distribution.

This study underscores the critical role of implant data type in ensuring the reliability and interpretability of biomechanical simulations. The findings provide valuable guidance for researchers and engineers working on patient-specific implant development and biomechanical analyses, highlighting the trade-offs between data detail and simulation fidelity.

REFERENCES. [1] E. Saillard *et al.*, "Finite element models with automatic computed tomography bone segmentation for failure load computation," *Sci Rep*, vol. 14, no. 1, Dec. 2024, doi: 10.1038/s41598-024-66934-w.

Po4_2.30

The Comparative Biomechanical Effects Of Remplissage And Dynamic Anterior Stabilization On Treating On-Track And Off-Track Bipolar Bone Loss

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Abstract

Purpose: To compare the biomechanical effects of remplissage and dynamic anterior stabilization (DAS) in the treatment of anterior shoulder instability with bipolar bone loss.

Methods: Eight fresh-frozen cadaveric shoulders were tested in the intact, injury, and repair conditions. Injury conditions included 15% glenoid bone loss with an on-track or off-track Hill-Sachs lesion. Repair conditions included isolated Bankart repair (BR), BR with remplissage, and BR with DAS. The glenohumeral stability was assessed by measuring the anterior translation under 0, 10, 20, 30, 40, 50 N load and maximum load without causing instability at mid-range (60°) and end-range (90°) external rotation (ER). Maximum range of motion (ROM) was measured by applying a 2.2-N·m torque in passive ER and internal rotation (IR).

Results: Isolated BR failed to restore native glenohumeral stability in both on-track and off-track bipolar bone loss models. Both remplissage and DAS significantly decreased the anterior instability in the bipolar bone loss models, showing better restoration than the isolated BR. In the on-track lesions, DAS successfully restored native glenohumeral stability and mobility, while remplissage significantly decreased anterior translation without load ($P = 0.003$ at 90° ER; $P = 0.015$ at 60° ER). In the off-track lesions, remplissage restored native glenohumeral stability but led to significant ROM limitation ($P < 0.001$ for IR; $P = 0.003$ for ER), whereas DAS failed to restore stability at 90° ER regarding the increased anterior translation under 50 N and decreased maximum load as compared with both the intact condition ($P < 0.016$ and $P = 0.021$) and remplissage ($P = 0.008$ and $P = 0.016$), respectively.

Conclusion: Both remplissage and DAS significantly reduced residual anterior instability of isolated BR in the bipolar bone loss models and restored the native glenohumeral stability under most translational loads. However, remplissage decreased the anterior translation without load for on-track lesions and restricted ROM for off-track lesions; whereas DAS failed to restore native stability under high translational loads for off-track lesions.

Clinical Relevance: DAS could be recommended to treat on-track bipolar bone loss with less biomechanical adverse effects, whereas remplissage might be the preferred procedure to address off-track bipolar bone loss for better stability.

Po4_2.40

Bilateral cervical and shoulder range of motion asymmetry in university para athletes with neurological disorders

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Abstract

Background: Neck pain is a common symptom in paralympic athletes and wheelchair users. Additionally, shoulder pain is common in wheelchair users, given the need to rely more on their upper limbs for mobility. Individuals with neurological disorders, such as spinal cord injury (SCI) and spina bifida often present with upper limb motor dysfunction that may likely impact range of motion. It remains unknown how active range of motion is affected in an athletic population of wheelchair users with neurological disorders.

Objective: To evaluate active cervical and shoulder range of motion asymmetry in a sample of university para-athletes with neurological disorders from the United States.

Methods: Nineteen para-athletes (male sex n=12, 63.2%; age= 22.37±2.5 years) completed an active cervical and shoulder range of motion assessment using a three-dimensional movement analysis system. Specific disorders consisted of SCI (n=10), spina bifida (n=4), transverse myelitis (n=2), and other (n=3). Motions in the analyses consisted of peak degrees (°) of cervical lateral flexion and rotation to the left and right, along with glenohumeral flexion, abduction, internal rotation and external rotation between the dominant and non-dominant limbs. Cervical flexion and extension were not included in the analyses due to no bilateral comparison. Glenohumeral adduction and extension were not included in the analyses due to not being recorded in the movement analysis system. A Shapiro-Wilk test for normality revealed that cervical rotation and glenohumeral flexion were not normally distributed and thus Wilcoxon Signed-Rank tests were used for these motions, while dependent samples t-tests were used for all other motions.

Results: Para-athletes had significantly greater cervical lateral flexion to the left (34.84°±5.3°) than right (26.79°±8.9°; p<0.001). No differences were observed between cervical rotation left (64.47°±14.9°) and right (67.95°±9.4°; p=0.11). No asymmetrical differences were observed on glenohumeral motions (p range = 0.07 [internal rotation]-0.66 [abduction]) between limbs.

Conclusion: Para-athletes displayed an 8° difference in active cervical lateral flexion asymmetry, that may be due to unilateral tight sternocleidomastoid, scalene, and trapezius musculature on their dominant side (17/19 participants were right arm dominant). Neurological disorders that primarily affect the spine may warrant special consideration in cervical soft tissue care and utilization of neuromuscular techniques to restore functional symmetry, which may also decrease injury risk and incidence.

Po4_3.01

A disruptive Organ-on-Chip device with a paper-based origami configuration: the Phoenix-OoC

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Abstract

Organ-on-chip (OoC) technology has emerged as a transformative tool in biomedical research, offering a platform that bridges the gap between conventional in vitro and in vivo models and the complexity of human physiology. These microengineered systems replicate the dynamic mechanical and biochemical environment of human organs, enabling more accurate studies of organ-level function and disease mechanisms. In this study, we present the development of an innovative OoC platform designed to model the knee joint microenvironment, with a specific focus on osteoarthritis (OA).

Our approach integrates human chondrocytes, adipose cells, synovial cells, and bone cells cultured within hydrogels engineered to mimic the extracellular matrix of the osteochondral unit. This biomimetic system recreates the knee joint's cellular heterogeneity and mechanical dynamics, providing a robust platform for studying OA, a degenerative joint disease characterized by cartilage degradation, synovial inflammation, and subchondral bone remodeling. The platform facilitates detailed investigations into OA pathophysiology and therapeutic interventions by replicating the knee joint's biochemical and biomechanical interactions.

To enhance the platform's functionality, we incorporate paper-based microfluidic analytical devices (μ PADs) in an origami-like configuration. These μ PADs are real-time sensors embedded within the OoC system, enabling continuous monitoring of cell behavior and responses to biochemical and mechanical stimuli. This real-time data acquisition capability enhances our understanding of cellular processes and supports more precise pharmacological studies.

The integration of μ PADs into OoC platforms advances organ modeling and aligns with sustainability goals, as paper-based devices offer an environmentally friendly alternative to traditional materials. The resulting system represents a significant step forward in creating versatile, accurate, and sustainable tools for biomedical research. This study highlights the potential of OoC technology to revolutionize drug discovery, reduce animal testing, and deepen insights into human physiology and pathology, with broad implications for personalized healthcare and sustainable innovation.

Acknowledgments. The authors thank Phoenix-OoC project, funded under European Union's Horizon Europe EIC 2023 Pathfinder Open program, grant agreement No 101130395.

Po4_3.02

Identification of Two Biological Subgroups of Complex Regional Pain Syndrome Type 1 by Transcriptomic Profiling of Skin and Blood

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Abstract

Background. Complex Regional Pain Syndrome (CRPS) is a challenging pain disorder often triggered by trauma, fractures, or surgical interventions, characterized by disproportionate pain and a cascade of neuroinflammatory and musculoskeletal changes. Proper diagnosis of CRPS is crucial, as it can significantly prevent the progression of trauma-related complications. This study aimed to identify the molecular signatures of potential CRPS Type 1 subgroups.

Methods. Twelve women with CRPS type 1 were included. Demographics and pain questionnaires were recorded. Skin biopsies of the affected and non-affected limbs (n=6+6) and peripheral blood (n=11) were collected. RNA sequencing was performed on skin and peripheral blood mononuclear cells (PBMCs). Twenty cytokines were quantified in blood plasma (n=12).

Results. Cluster analysis of the affected skin identified two CRPS subgroups (SG). SG1 exhibited increased gene expression related to epidermal development, metabolic processes, and a greater abundance of keratinocytes (Figure 1A, 1B). SG2 showed enhanced transcriptomic changes in inflammatory, immune, and fibrotic processes, along with a higher abundance of fibroblasts, macrophages, and endothelial cells (Figure 1A, 1B). PBMCs transcriptomics revealed the same SG1/SG2 clusters (Figure 2A) and highlighted a stronger inflammatory response in the blood of SG1, suggesting distinct tissue-specific immune responses for the subgroups. Interleukin-1 receptor antagonist (IL-1RA) levels were higher in the blood plasma of SG1 (FDR=0.01) (Figure 2B), consistent with its encoding gene *IL1RN* expression in PBMCs (log2 FC=1.10, $P<0.001$) and affected skin (log2 FC=0.88, $P=0.006$). Subgroups did not differ in demographic or clinical parameters but correlations among clinical factors varied between them.

Conclusions. This study identified two biological subgroups of CRPS type 1 through skin and blood transcriptomic profiling, advancing the understanding of this condition. These

insights could enhance the precision of interventions, ultimately improving patient outcomes.

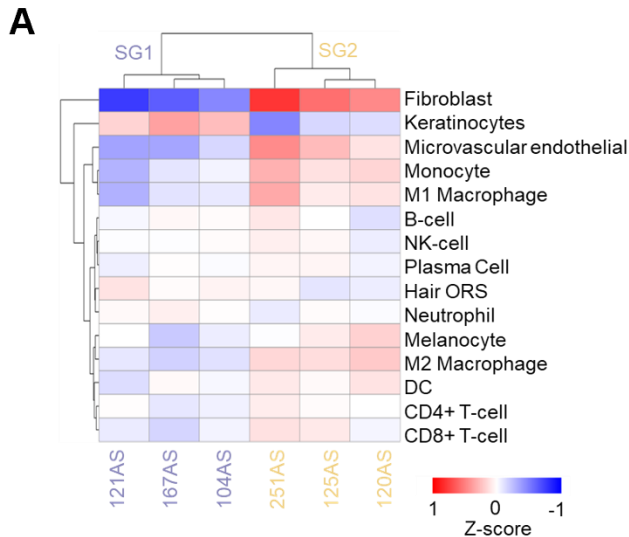


Figure 1. (A) Deconvolution analysis of AS displaying patient stratification according to the cell signature score. (B) Gene Set Enrichment Analysis (GSEA) of hallmark gene sets comparing SG2 and SG1

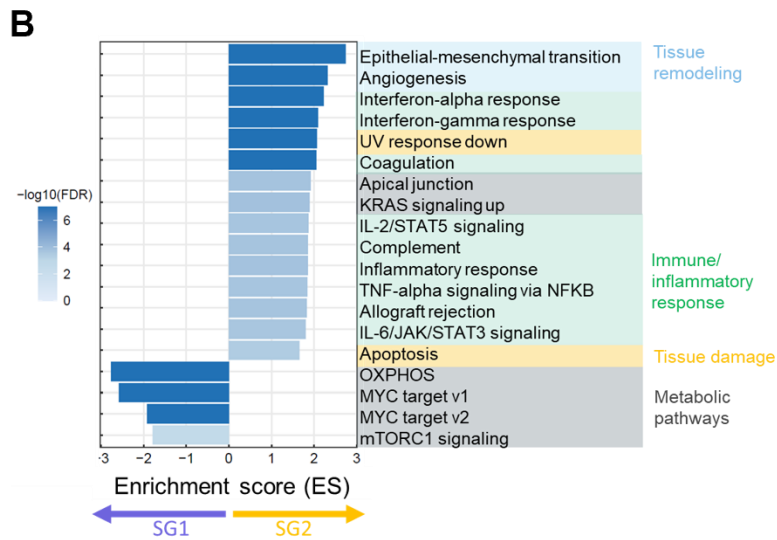
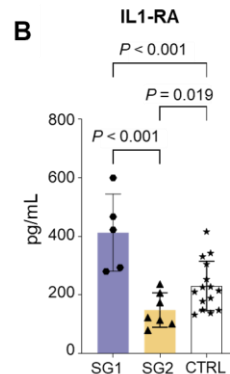
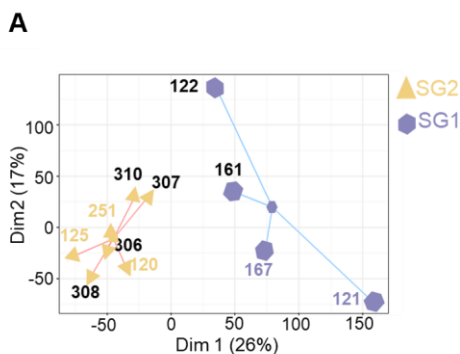


Figure 2. (A) K-means clustering of normalized transcriptomic data from 11 PBMCs CRPS samples. (B) IL-1RA plasma levels in SG1 (n=5), SG2 (n=7), and healthy volunteers (CTRL, n=15). ANOVA followed by Tukey Post-hoc test.



Po4_3.04

Does Metabolomic Profile Differ in Conventional and Activated Platelet-Rich Plasma?

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Abstract

Platelet-Rich Plasma (PRP) is widely used for its anti-inflammatory and regenerative effects in musculoskeletal conditions. Activation with chemical mediators like calcium chloride or thrombin enhances the release of growth factors such as PDGF, TGF- β , and VEGF. This study evaluates metabolomic differences between conventional and activated PRP types using Q-TOF LC/MS.

Blood samples (140 mL) from six male volunteers were processed to obtain leukocyte-rich (L-PRP), leukocyte-poor (P-PRP), and three activated PRP types (PRGF, active PRP G, active PRP S). Metabolites were extracted via protein precipitation with methanol, dried, and reconstituted in an acetonitrile-water solution (50:50). Mass spectrometry was conducted in negative ion mode (m/z 75–1200), and raw data were processed with MZmine and analyzed using MetaboAnalyst 6.0, referencing HMDB and KEGG databases.

Principal Component Analysis (PCA) demonstrated clear distinctions among PRP types. Heatmaps revealed metabolic consistency within groups and highlighted specific metabolites that vary across conditions, indicating significant metabolomic differences between PRP types.

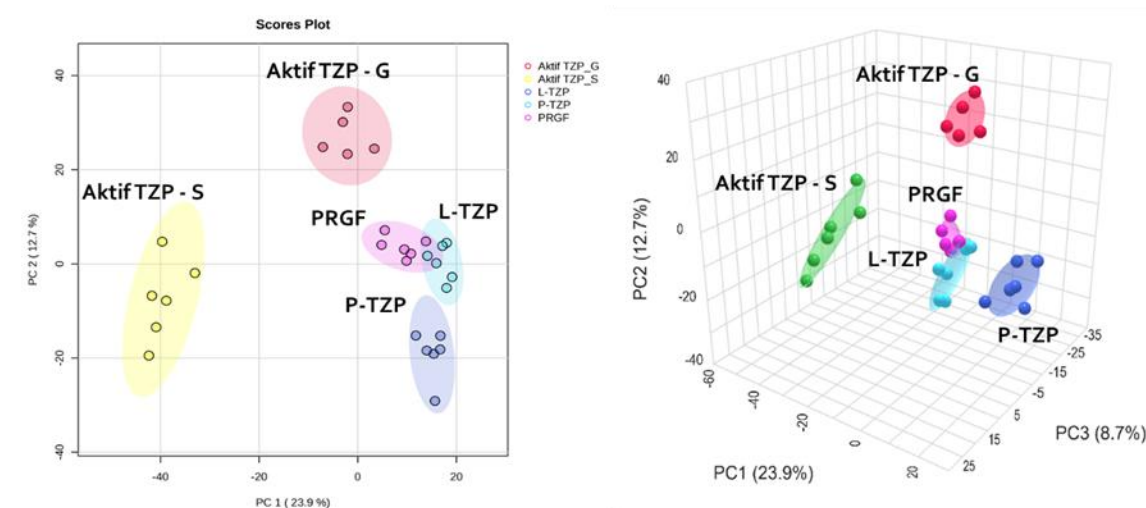


Figure 1. PCA plots of PRP types (Left: 2D, Right: 3D).

In the heatmap (Figure 2), the rows with the most intense red or blue coloration correspond to metabolites displaying the greatest magnitude of variation across the groups, thereby indicating where intergroup differences are most pronounced.

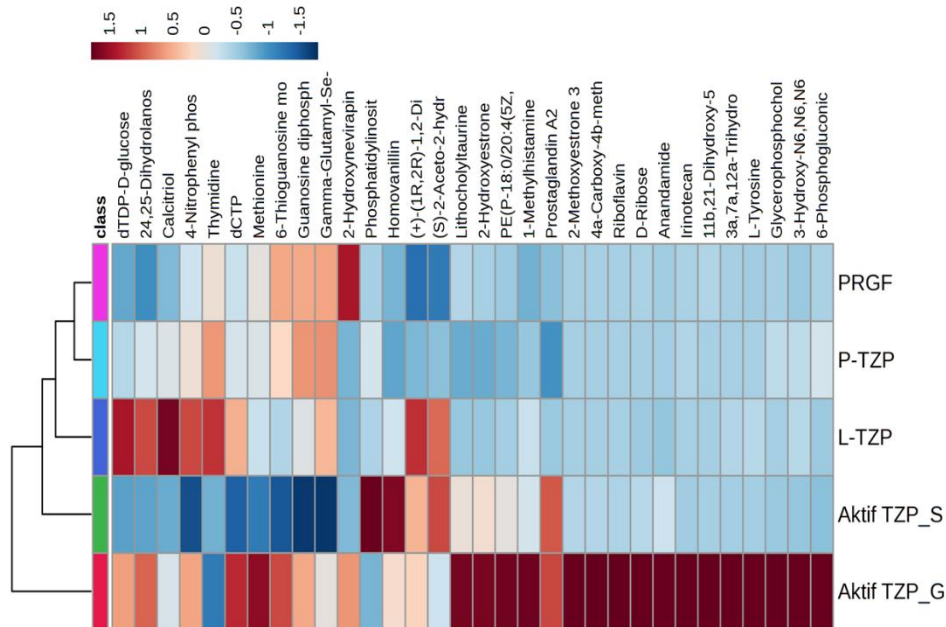


Figure 2. Comprehensive heatmap illustrating metabolite intensity distributions across different PRP groups.

Phospholipids in the platelet membrane structure (e.g., phosphatidylinositol, phosphatidylethanolamine) and D-Glucose, which is involved in energy metabolism, play a critical role in platelet activation and the release of growth factors. Prostaglandins, belonging to the eicosanoid family (particularly Thromboxane A2, though Prostaglandin A2 is also involved in a related pathway), are essential in regulating platelet aggregation and, consequently, the hemostatic and healing response. The intermediate of the pentose phosphate pathway (PPP), 6-Phosphogluconic acid, contributes to maintaining the redox balance of platelets and meeting their energy demands through NADPH production.

A more in-depth examination of these metabolites may significantly improve the clinical efficacy of PRP, enabling the development of personalized treatment protocols that account for individual patient variations. Such optimization of metabolic components could accelerate tissue regeneration, mitigate complications during healing, and ultimately enhance patient outcomes within a personalized medicine framework.

Po4_3.06

Arthrodesis of the subtalar joint using a novel biphasic calcium phosphate bone graft

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Abstract

Study design. Retrospective level IV study.

Background. Limited availability of autologous bone graft has led to a vast array of alternative bone graft options including allograft, demineralized bone matrices, cell-based matrices, and synthetic bone grafts. In this retrospective review, a control group of patients undergoing subtalar joint arthrodesis using a conventional bone graft was compared to a matched study group utilizing a novel synthetic biphasic calcium phosphate (BCP) bone graft with advanced surface topography.

Materials and methods. Seventeen consecutive patients underwent subtalar joint arthrodesis using a novel BCP with a unique submicron surface topography, either as a standalone graft or mixed with bone marrow aspirate. Fusion outcomes were assessed via radiographs at six-weeks and twelve-weeks. Clinical outcomes were assessed via weight bearing status at six-weeks and twelve-weeks. These results were compared to a matched study group of 15 patients undergoing subtalar joint arthrodesis using a conventional bone graft.

Results. Seventeen of seventeen (100%) of patients completed full follow up. Twelve of seventeen patients had complete fusion at 12 weeks (70%) and four of seventeen patients (23%) had partial fusion at 12 weeks. One of seventeen (5%) had revision arthrodesis. All patients were 100% weight bearing at 12 weeks. In the control group, fifteen of fifteen (100%) of patients completed full follow up. At 12 weeks, seven of fifteen (46%) had complete fusion, seven of fifteen (46%) had partial fusion, and one (6%) did not fuse and went on to revision surgery.

Conclusions. This novel submicron surface topography BCP offers a promising bone graft substitute for arthrodesis of the subtalar joint.

Po4_3.07

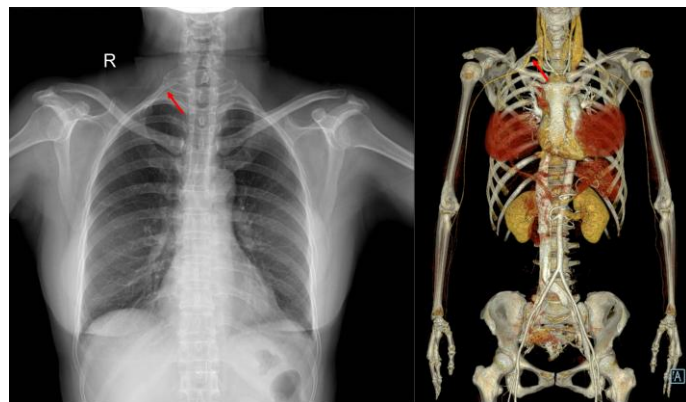
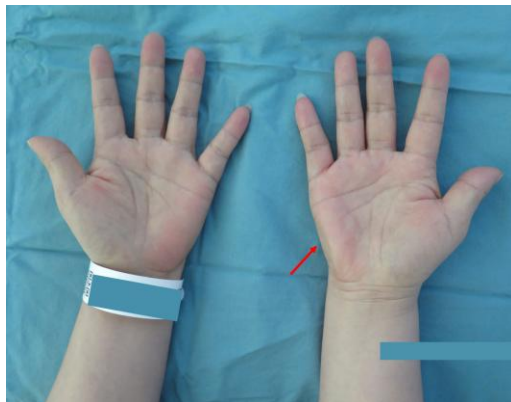
A Mahjong Player's Hidden Handicap

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Abstract

A 53-year-old woman presented to the orthopaedic surgery clinic with a two-year progression of numbness and weakness along the medial side of her right forearm and the ulnar side of her hand. Her symptoms worsened over the past month because of extensive periods of playing mahjong. Clinical examination revealed sensory deficits along the ulnar side of the right forearm, intrinsic muscle wasting in the right hand, decreased grip strength, along with diminished radial artery pulsation. Significant tenderness was found in the right supraclavicular fossa with forearm radiating pain. Positive Adson's test and Cervical Rotation Lateral Flexion test further indicated her diagnosis. Imaging revealed a right seventh cervical rib compressing the subclavian artery and brachial plexus, directly contributing to her symptoms. The presence of a cervical rib, alongside with her typical ischemia and never compression symptoms, led to the diagnosis of mixed neurogenic and arterial thoracic outlet syndrome, which was a rare and complex presentation. She received surgical remove of the right seventh cervical rib to alleviate the vascular and neurogenic compression. Postoperatively, the patient experienced significant relief from her symptoms and showed good recovery. This is a relatively rare case of the thoracic outlet syndrome, predominantly



featuring neurogenic symptoms along with symptoms of arterial compression. The neurogenic component was indicated by the muscle wasting and sensory deficits, while the arterial component was evidenced by the vascular compression seen in imaging, reduced radial pulse, chilliness of the affected limb.

Po4_3.09

Vascular invasion model of hypertrophic cartilage-like pellets: An *in vitro* feasibility study.

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Abstract

Background: Bone is highly regenerative, but 5-10% of fractures result in non-unions, they are often associated with impaired vasculature [1]. However, some non-union cases are well vascularized. Hence, adequate vascularization is not the full answer [2]. Understanding cellular interactions during endochondral bone healing would lead to better therapies to avoid, or rescue healing failures. In this preliminary study, we produced and characterized TGFβ1 pre-treated cellular pellets that showed microvascular invasion potential.

Materials and Methods: Cellular pellets (50,000cells/pellet) were generated from human bone marrow-derived mesenchymal stem cells (MSCs) (KEK ZH NR 2010 0444) labelled with PKH26 red fluorescent dye and cultured in chondrogenic (TGFβ1) medium for 7 days. Chondrogenic differentiation of the pellets was assessed through qRT-PCR, histological analysis and sGAG quantification. These pellets were then cultured in direct co-culture with GFP-HUVECs and pericytes in a 9/1 ration, in low-stiffness Lunagel (Gelomics) containing Ibidi chambers. The co-culture systems were cultured in presence of angiogenic M200 medium for 17 days. Micro vascular invasion was assessed at different time points using confocal laser scanning microscopy.

Results: Data showed vascular invasion of the cellular pellets (Figure 1), by day 17 of co-culture. qRT-PCR analysis showed an upregulation of COL10A1 and COL2A1 gene expression at Day7 compared to Day1 (Figure 2 A). No substantial upregulation was observed for MMP13 and IHH. The cellular pellets characterization showed an increase in the glycosaminoglycans content as shown by the Safranin-O/Fast green staining and sGAG quantification results (Figure 2 B and C).

Conclusion: After 7 days in chondrogenic medium, cellular pellets showed a strong up regulation of both type 10 and type 2 collagen, compatible with and early hypertrophic-chondrocyte differentiation stage [3] while other hypertrophic markers like MMP13 and IHH were less regulated. Further investigations, including more donors, are required to conclusively characterize the phenotype of the cellular pellets and their influence on the vascular invasion process.

Analysis of conditioned media at different time points is currently in process. Additionally, the pellets after day 17 of co-culture should also be characterized.

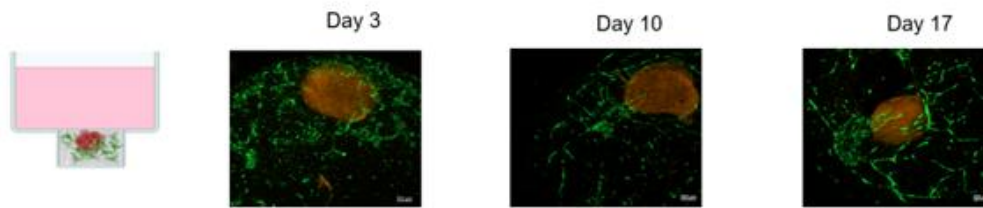


Figure1: Direct co-culture of MSC pellets and HUVEC-PC showed signs of micro-vascular network formation after day 3 and signs of pellet's invasion after 17 days in culture.

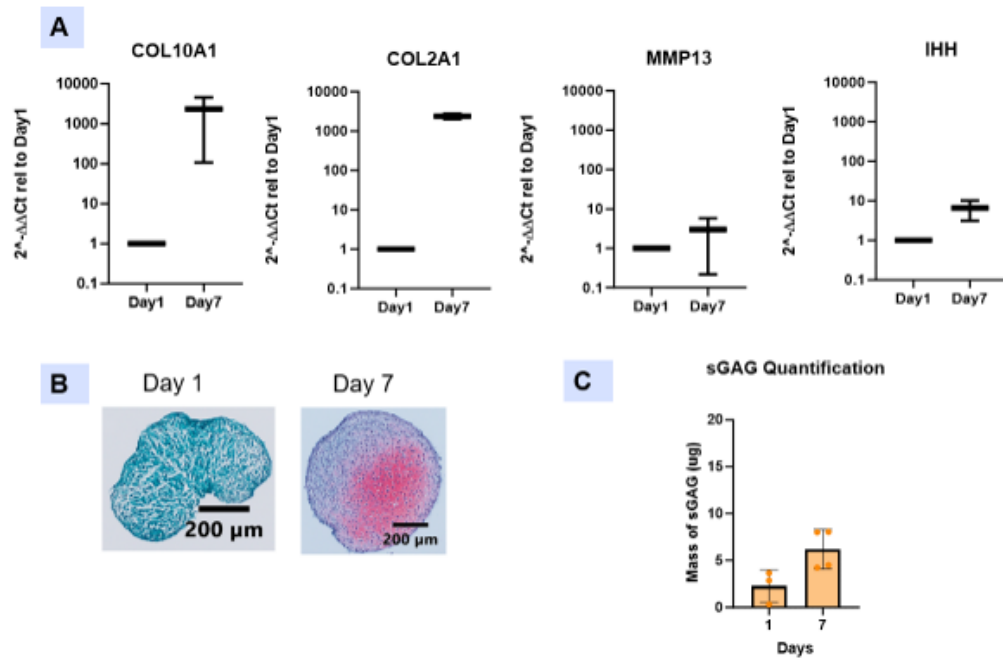


Figure2: (A) shows the gene expression results of cellular pellets in chondrogenic condition, substantial upregulation of COL10A1 and COL2A1 is observed at day7. (B) shows the Safranin-O-Fast-Green staining, with higher proteoglycan deposition by day 7. (C) shows the sGAG quantification of the cellular pellets, with increase in day 7.

References: [1] Holmes et al, Nature, 2017; [2] Menger et al, Angiogenesis, 2022; [3] Scotti et al, PNAS, 2010.

Po4_3.10

Does Freeze-Thawing Change the Metabolomic Active of Platelet-Rich Plasma?

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Abstract

Platelet-rich plasma (PRP) is often frozen and thawed for repetitive clinical use. This study examines whether freezing and thawing alter PRP's metabolomic activity. Active PRP, prepared using activators like calcium chloride or thrombin, releases growth factors such as PDGF, TGF- β , and VEGF. In contrast, conventional PRP relies on natural activation at the application site. The metabolomic differences between active and conventional PRP types were investigated.

Blood from six male volunteers was used to prepare two conventional PRP types (leukocyte-rich [L-PRP] and leukocyte-poor [P-PRP]) and three active PRP types (PRGF, active PRP G, and active PRP S). Samples were prepared fresh or stored at -20°C for one week. After protein precipitation and metabolite extraction, samples were analyzed using Q-TOF LC/MS. Data were processed with MZmine and MetaboAnalyst 6.0, and metabolite identities were confirmed using HMDB and KEGG databases.

Metabolomic analysis revealed significant changes in all PRP types after freezing and thawing, except for active PRP-S (Figure 1). L-PRP showed the most differentiation, with over 250 peaks altered after freezing. Key metabolites like glucose, phosphocreatine, and citric acid, crucial for energy metabolism, decreased across PRP types. Antioxidants such as glutathione and biliverdin also declined, potentially compromising PRP's regenerative capacity and increasing oxidative stress.

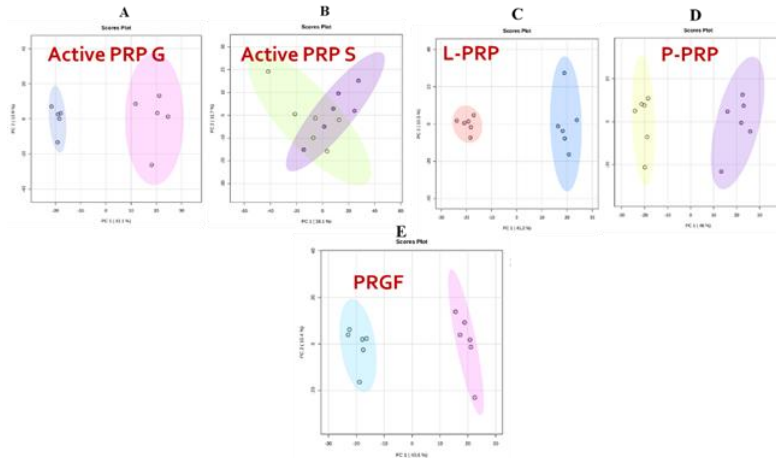


Figure 1. PCA plots for fresh and frozen PRP samples.

The freeze-thawing process led to a general decrease in metabolites, indicating a lack of stability. Significant reductions were observed in energy metabolism components (glucose, phosphocreatine, citric acid) and antioxidant defense systems (glutathione, biliverdin). This suggests that frozen PRP may have a lower regenerative capacity compared to fresh PRP. Furthermore, when considering the aspects of degeneration and inflammation, the decrease in metabolites and antioxidants associated with oxidative stress may lead to an increase in inflammatory processes or make their control more challenging. The reduction in neurotransmitter derivatives such as dopamine and catecholamine metabolites like 3,4-dihydroxyphenylglycol may also impact the regulation of immune and inflammatory responses.

In conclusion, the metabolomic differences between various PRP types are significant in terms of both clinical efficacy and storage stability. Selecting the appropriate PRP type tailored to the patient's needs and the targeted therapeutic effect, as well as optimizing storage conditions, can improve treatment outcomes in clinical applications. This study sheds light on the storage stability of different PRP types, contributing to the development of effective treatment plans in PRP applications for physicians.

Po4_3.11

Mechanoadaptation and tumor stemness driven by O-GlcNAcylation of the Notch1 intracellular domain promote stiffness-resistance and chordoma recurrence

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Abstract

Chordoma, an extremely rare malignant bone tumor, is characterized by high rate of recurrence. As observed clinically, the extracellular microenvironment of recurrent tumor has higher stiffness compared to that of the primary tumor. We found that recurrent cells exhibit increased stress fiber, focal adhesions, and proteoglycan. We propose a novel model of chordoma recurrence where cells mechanically adapt to stiff microenvironment, facilitating resistance to high stiffness-induced cell death. Central to this process is the O-GlcNAcylation of the Notch1 intracellular domain (NICD1). Mechanically, the ligand-independent phosphorylation of EPHA2 in response to stiff ECM activates LYN kinase, and subsequently triggers OGT activity by phosphorylating Y989 and Y418, the newly revealed critical residues for OGT glycosyltransferase activity, contributing to O-GlcNAcylation of NICD1 T2063, T2090 and S2162 residues, which lead to specifically transcribing mechanical and tumor stemness-related genes. *MIR31* deletion in recurrent chordoma upregulates LYN, which promotes perceiving stiffness and tips the balance toward O-GlcNAc addition, suggesting its potential as a biomarker for indicating recurrence and patient stratification in Notch- or OGT-targeted therapy.

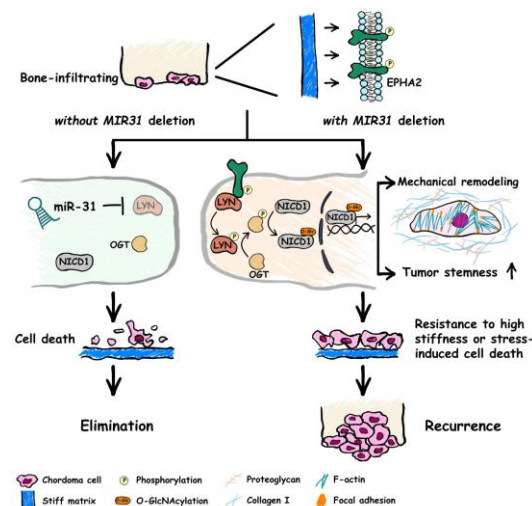


Figure 1. The schematic diagram showing the proposed process of chordoma development, during which MIR31 deletion-induced upregulation of LYN promotes chordoma cells to adapt to ECM microenvironment with high stiffness.

Po4_3.12

Expression and therapeutic potential of CDK11 in chordoma

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Abstract

Chordomas are rare and generally slow-growing invasive tumors that lack an effective form of drug treatment. Currently, surgical resection remains the primary form of intervention, however this is made extremely difficult given this malignancy's tendency to localize near important anatomical structures. Conventional chemotherapy and radiotherapy remain inadequate forms of treatment, while targeted therapies and immunotherapies also prove widely unsuccessful. Ultimately, there exists a critical need for the development of a novel therapeutic strategy that can improve chordoma patient outcome.

Cyclin-dependent kinase 11 (CDK11) has proven to be an essential gene for the growth of several malignancies. The overexpression of CDK11 has been associated with various types of tumors and often leads to poor clinical outcome. Currently, the association between the expression of CDK11 and its clinical significance in chordoma remains unknown. Thus, the objective of this study was to detect the expression of CDK11 in chordoma and determine its therapeutic implications. In this study we evaluated the expression of CDK11 in chordoma cell lines and tissues using Western blot analysis and immunohistochemistry. Western blot analysis confirmed that CDK11 is highly expressed and activated in several chordoma cell lines and in resected chordoma tumor samples. Immunohistochemistry analysis showed that the majority of chordoma tissue analyzed (97.7%) expressed CDK11. These data also revealed that high CDK11 expression correlated with metastasis and recurrence of chordoma. In this study we also investigated the functional role of CDK11 in chordoma cell growth and proliferation, while also determining its therapeutic implication via inhibition with the use of a CDK11 inhibitor known as OTS964. Treatment of chordoma cells using this inhibitor showed that OTS964 can efficiently inhibit the growth and proliferation of chordoma cells in the cell lines UCH1 and CH22. A combination assay utilizing OTS964, and doxorubicin was also performed. This assay determined that OTS964 can increase doxorubicin drug sensitivity. Accordingly, usage of both inhibitors for the treatment of chordoma may prove useful if further investigated. These data help highlight the association between CDK11 expression and chordoma growth and proliferation. Given that CDK11 plays a critical role in the regulation of pre-mRNA splicing, these data showcase the dependency of cancer cells on CDK11. Ultimately, this study presents CDK11 as a novel therapeutic target for the treatment of chordoma.

Po4_3.13

Decreased EZH2 expression impairs mitosis and cell cycle process through noncanonical mechanisms in senescent NPMSCs: Based on RNA-seq and CUT&Tag

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Abstract

INTRODUCTION: Intervertebral disc degeneration (IVDD), a major cause of low back pain, involves cellular senescence of nucleus pulposus mesenchymal stem cells (NPMSCs) due to unfavorable factors. EZH2 is an epigenetic regulatory factor closely related to maintenance of stemness and cell differentiation. However, the downstream network of EZH2 in senescent NPMSCs remains unclear. The aim of this study was to investigate the expression changes of EZH2, identify potential target genes for EZH2, and elucidate its regulatory mechanisms in senescent NPMSCs.

METHODS: The EZH2 expression of NPMSCs in human nucleus pulposus samples and rat models was measured by multiplex immunohistochemistry (mIHC). Real-time quantitative PCR (RT-qPCR) and Western blot were performed to assess the expression of EZH2 in vitro senescent human NPMSCs. RNA-seq and CUT&Tag were performed to analysis the target genes of EZH2. Finally, mIHC was performed to verify the expression changes of EZH2 and hub genes in human nucleus pulposus tissue.

RESULTS: Histological and imaging analyses revealed intervertebral disc narrowing, reduced MRI signal intensity, and loss of proteoglycans in both human and rat IVDD (Figure 1 A, B). Concurrently, a decrease in EZH2 expression was observed in aging NPMSCs (Figure 1 C, D). Decreased expression of EZH2 was also observed in vitro senescent NPMSCs (Figure 2 A-C). Then, the cross analysis of RNA-seq and CUT&Tag results showed that 58 target genes were directly regulated by EZH2 (Figure 2 D, E). The protein-protein interaction network analysis identified the hub genes associated with the mitotic cell cycle and cell proliferation, including TTK, MCM6, AURKB, NCAPG, and CDCA8 (Figure 3 F, G). Finally, mIHC results verified the decreased expression of EZH2 and hub genes in IVDD (Figure 2 H).

DISCUSSION: In both human and rat IVDD, as well as in vitro oxidative stress-induced cell senescence model, our study found a reduced expression of EZH2 in senescent NPMSCs. Based on RNA-seq and CUT&Tag, this study demonstrated that EZH2 regulates the expression of hub genes through its noncanonical function in NPMSCs. These hub genes are related with the mitotic cell cycle and cell proliferation. Therefore, EZH2 may be a potential therapeutic target for IVDD, but its transcriptional regulatory functions in NPMSCs still require further investigation.

SIGNIFICANCE: Our research revealed potential mechanisms through which EZH2 influences NPMSCs senescence, providing theoretical support for considering EZH2 as a potential therapeutic target for IVDD.

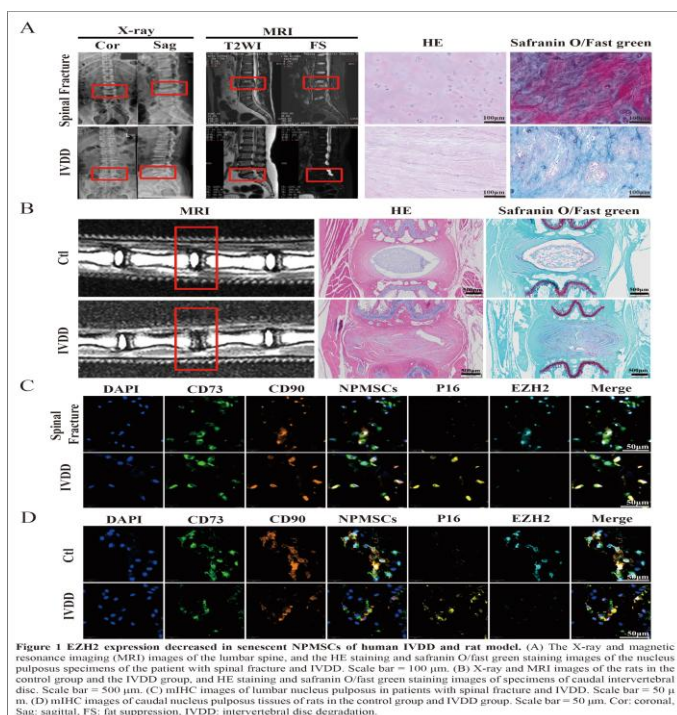


Figure 1 EZH2 expression decreased in senescent NPMSCs of human IVDD and rat model. (A) The X-ray and magnetic resonance imaging (MRI) images of the lumbar spine, and the HE staining and safranin O-fast green staining images of the nucleus pulposus specimens of the patient with spinal fracture and IVDD. Scale bar = 100 μ m. (B) X-ray and MRI images of the rats in the control group and the IVDD group, and HE staining and safranin O-fast green staining images of specimens of caudal intervertebral disc. Scale bar = 500 μ m. (C) mIHC images of lumbar nucleus pulposus in patients with spinal fracture and IVDD. Scale bar = 50 μ m. (D) mIHC images of caudal nucleus pulposus tissues of rats in the control group and IVDD group. Scale bar = 50 μ m. Cor: coronal, Sag: sagittal, FS: fat suppression, IVDD: intervertebral disc degeneration.

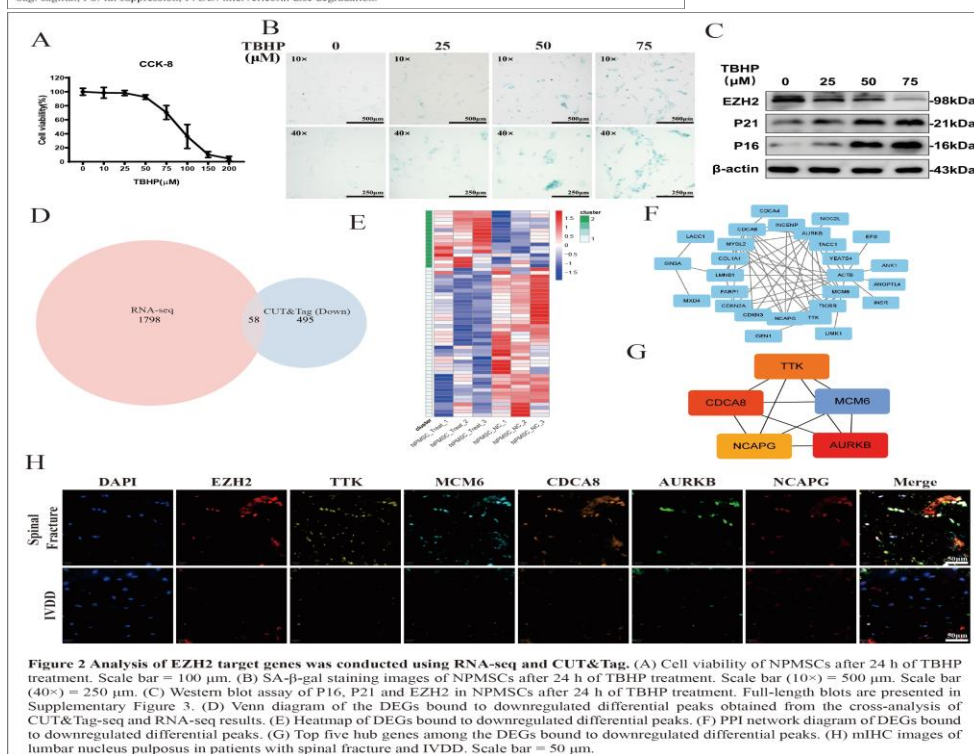


Figure 2 Analysis of EZH2 target genes was conducted using RNA-seq and CUT&Tag. (A) Cell viability of NPMSCs after 24 h of TBHP treatment. Scale bar = 100 μ m. (B) SA- β -gal staining images of NPMSCs after 24 h of TBHP treatment. Scale bar (10 $\times$) = 500 μ m. Scale bar (40 $\times$) = 250 μ m. (C) Western blot assay of P16, P21 and EZH2 in NPMSCs after 24 h of TBHP treatment. Full-length blots are presented in Supplementary Figure 3. (D) Venn diagram of the DEGs bound to downregulated differential peaks obtained from the cross-analysis of CUT&Tag-seq and RNA-seq results. (E) Heatmap of DEGs bound to downregulated differential peaks. (F) PPI network diagram of DEGs bound to downregulated differential peaks. (G) Top five hub genes among the DEGs bound to downregulated differential peaks. (H) mIHC images of lumbar nucleus pulposus in patients with spinal fracture and IVDD. Scale bar = 50 μ m.

Po4_3.16

Tocopherol attenuates IVDD by inhibiting ferroptosis in nucleus pulposus mesenchymal stem cells

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Abstract

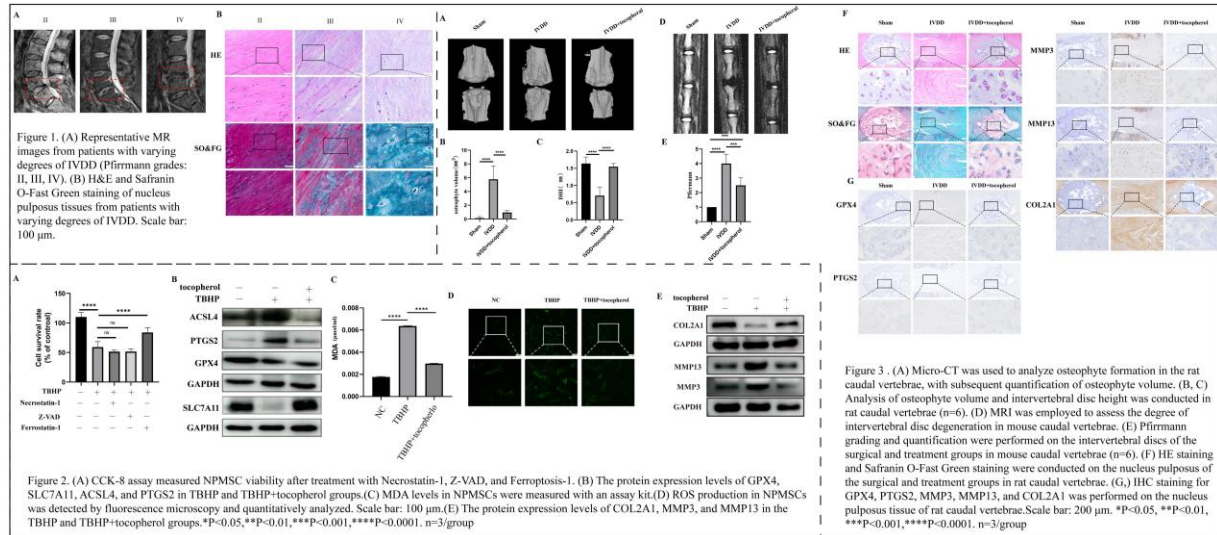
INTRODUCTION: Intervertebral disc degeneration (IVDD) is a common spinal disorder that significantly impacts quality of life. Understanding the pathological mechanisms of IVDD is essential for developing new treatments. Studies suggest that lipid peroxidation of polyunsaturated fatty acids (PUFAs) and ROS accumulation, leading to ferroptosis, contribute to IVDD. Tocopherol, a potent antioxidant, may mitigate ROS-induced membrane damage by inhibiting lipid peroxidation. However, it remains unclear whether tocopherol can effectively prevent IVDD by inhibiting ferroptosis.

METHODS: According to MRI detection, nucleus pulposus tissues from intervertebral disc degeneration patients were categorized into grades II, III, and IV, followed by HE and SO&FG staining. NPMSCs were treated with tocopherol for 24 hours and then used TBHP induced ferroptosis, RT-qPCR and WB were used to analyze ferroptosis markers and ECM expression changes, alongside ROS and MDA levels. In animal model, rats were divided into sham, model, and treatment groups. Four weeks post-surgery, caudal vertebrae were collected for MRI and Micro-CT analysis. HE and SO&FG staining were then performed, with ferroptosis and ECM markers analyzed through IHC.

RESULTS SECTION: MRI and histological staining confirmed NP tissue degeneration in the intervertebral disc (Figure 1A, 1B). In vitro, tocopherol pretreatment inhibited the downregulation of GPX4 and SLC7A11 and prevented the upregulation of PTGS2 and ACSL4 compared to the TBHP group. Similar results were observed in ROS and MDA experiments (Figure 2B-D). Tocopherol also reduced TBHP-induced COL2A1 degradation and inhibited MMP3 and MMP13 upregulation (Figure 2E). In vivo, micro-CT and MRI results revealed that tocopherol effectively reduced osteophyte formation and slowed both disc degeneration and nucleus tissue deterioration compared to the model group (Figure 3A-E). HE and SO&FG staining revealed that tocopherol significantly attenuated nucleus pulposus tissue degeneration compared to the model group (Figure 3F). IHC showed that tocopherol reversed the expression of GPX4, COL2A1, PTGS2, and ECM compared to the model group (Figure 3G).

DISCUSSION: Ferroptosis, an iron-dependent lipid peroxidation-induced cell death mechanism, is a critical factor in IVDD progression. Oxidative stress, closely linked to ferroptosis, is a key focus of IVDD research. This study confirmed ferroptosis in IVDD at tissue and cellular levels and explored tocopherol's therapeutic potential. Tocopherol

pretreatment of NPMSCs significantly reduced ferroptosis and ECM degradation. In vivo, tocopherol treatment markedly improved IVDD in rats, suggesting that targeting ferroptosis could offer new therapeutic approaches. However, further exploration of the mechanisms underlying tocopherol's effects on IVDD is needed.



Po4_3.17

Mechanisms of macrophage polarization toward M1 inhibition and delay of hormonal femoral head necrosis by EZH2 inhibitors through H3K27me3 modulation of SOCS3/TLR4 signaling

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Abstract

INTRODUCTION: Research indicates that osteonecrosis of the femoral head (ONFH) caused by factors such as hormones and alcohol is closely related to macrophage-mediated bone immunity. The continuous polarization of macrophages toward the M1 phenotype is a key factor in causing local chronic inflammation and ONFH. Recent studies have discovered that EZH2 regulates macrophage polarization to the M1 phenotype through H3K27me3 modification. The SOCS3/TLR4/NF- κ B pathway is one of the pathways regulated by EZH2, and it is closely associated with M1 polarization. However, it remains unclear whether the EZH2 inhibitor (GSK-126) can suppress M1 polarization and alleviates ONFH.

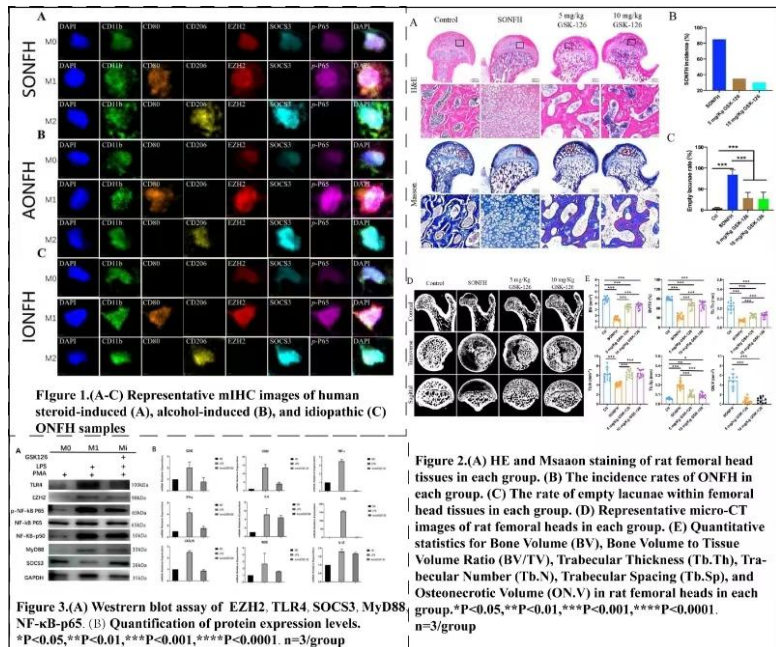
METHODS: In human samples of steroid-induced, alcohol-induced, and idiopathic ONFH, mlHC was employed to detect the expression of EZH2, SOCS3, and p-p65 in macrophages. In the animal experiment, the groups are categorized as Control, ONFH, ONFH + 5mg/kg GSK-126, ONFH + 10mg/kg GSK-126. Micro-CT and histological staining were performed to assess ONFH. Macrophages were treated with LPS to induce M1 polarization in vitro, followed by GSK-126 treatment to assess its effects on M1 macrophages. WB and RT-qPCR were utilized to evaluate the expression of M1 macrophage markers (CD80, TNF- α , IFN- γ , CCL2), EZH2, SOCS3, and components of the TLR4/NF- κ B signaling pathway (TLR4, MyD88, p65, p-p65).

RESULTS: mIHC analysis in ONFH revealed M1 macrophages exhibited high levels of EZH2 and p-p65, with no detectable SOCS3 expression. In vitro, treatment with the GSK-126 significantly reduced H3K27me3 levels in M1 macrophages, alleviating SOCS3 suppression and consequently inhibiting TLR4/NF- κ B signaling pathway activation. Imaging and histological assessments further demonstrated that GSK126 effectively mitigated the progression of femoral head

necrosis in a rat model. In vivo, through RT-qPCR and WB analysis, we found that GSK-126 promotes SOCS3 expression while inhibiting TLR4/NF- κ B expression.

Discussion: Our study delineates that in M1 macrophages across various types of ONFH, EZH2 is highly expressed, SOCS3 is downregulated, and the TLR4/NF- κ B pathway is activated. The EZH2 inhibitor (GSK-126) effectively alleviates rat ONFH, potentially by modulating macrophage polarization through the SOCS3/TLR4/NF- κ B pathway. However, the exact mechanism through which EZH2 controls macrophage polarization to the M1 phenotype via epigenetic regulation remains unknown, and we aim to investigate this in future research. However, further investigation is needed to elucidate which signaling pathways EZH2 regulates through its epigenetic or non-classical mechanisms to affect macrophage polarization.

SIGNIFICANCE: Our research suggests that EZH2 could be a potential therapeutic target for SONFH, providing novel insights and strategies for its treatment.



Po4_3.18

EZH2 regulates the NPMSC senescence via cGAS/STING signaling pathway through H3K27me3 and alleviates

Zhen Tan, [Jianrui Zheng](#), Zetao Ma, Deli Wang

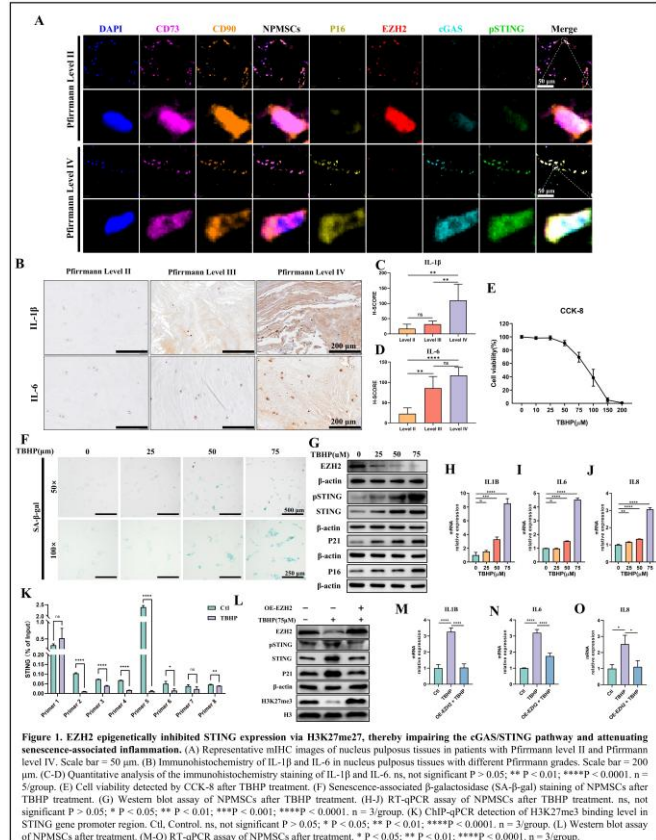
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Abstract

INTRODUCTION: Senescence of nucleus pulposus mesenchymal stem cells (NPMSCs) and associated inflammation promote the progression of intervertebral disc degeneration (IVDD). EZH2 is an epigenetic factor associated with the cellular senescence process and stemness maintenance. The cGAS/STING pathway senses damaged DNA within the Senescence cell and promotes senescence-associated inflammation. However, whether EZH2 can modulate senescence-associated inflammation through regulation of the cGAS/STING pathway by H3K27me3 remains to be further elucidated.

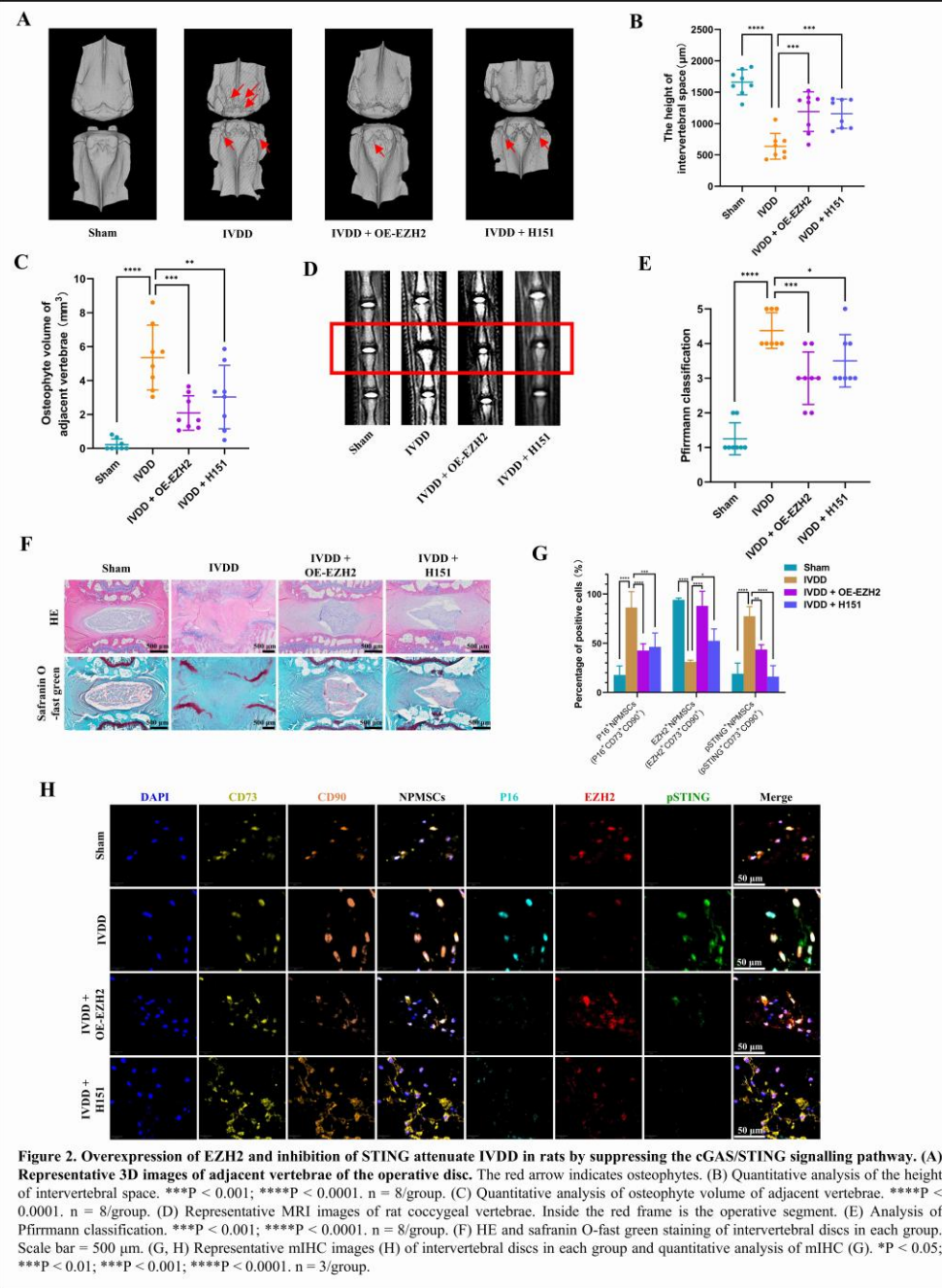
METHODS: In human nucleus pulposus tissues, Multiplex immunohistochemistry (mIHC) was conducted to detect the expression of EZH2 and cGAS/STING pathway. An in vitro model of NPMSCs senescence was constructed using TBHP. Subsequently, RT-qPCR, western blot, chromatin immunoprecipitation (ChIP) and lentivirus-overexpression-EZH2 (Lv-OE-EZH2) were employed to elucidate the effect of EZH2 on the cGAS/STING pathway and senescence-associated inflammation. In the animal experiment, the groups were categorized as sham, IVDD, IVDD + Lv-OE-EZH2, and IVDD + H-151 (a STING inhibitor). Micro-CT, MRI, and histological staining were performed to assess IVDD.

RESULTS: mlHC results revealed the presence of senescent NPMSCs in human degenerative nucleus pulposus tissues, characterized by decreased EZH2 expression and activation of the cGAS/STING pathway. (Figure 1 A-D). As the concentration of TBHP increases, the number of SA- β -gal positive NPMSCs increased, accompanied by elevated expression of P16 and P21, reduced EZH2 expression, and increased levels of STING and p-STING. (Figure 1 E-J). Utilizing ChIP assays and Lv-OE-EZH2, we validated that EZH2 epigenetically inhibited the expression of STING via H3K27me27, thereby impairing the cGAS/STING pathway and attenuating inflammation. (Figure 1 K-O). Additionally, the overexpression of EZH2 and inhibition of STING effectively mitigated osteophyte formation, intervertebral space narrowing, and reduced disc signal intensity by suppressing the cGAS/STING pathway (Figure 2).



DISCUSSION: In this study, we demonstrated that EZH2 expression decreased while the cGAS/STING pathway became activated in senescent NPMSCs both in human nucleus pulposus tissues and in vitro. Furthermore, the study found that Lv-OE-EZH2 and H-151 inhibited senescence-associated inflammation and attenuated rat IVDD. The cGAS/STING pathway has also been confirmed in other studies as a key driver of inflammation. Our research was particularly significant in demonstrating that EZH2 can modulate the cGAS/STING pathway, presenting a potential therapeutic target for IVDD.

SIGNIFICANCE: Our study unveils a pivotal role for EZH2 in the pathogenesis of IVDD. Our study verified the potential of EZH2 as a promising therapeutic target for IVDD.



Po5_1.04

Reevaluating Patient Isolation for Post-Operative Infections: a scoping review

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Abstract.

The isolation of patients with infectious diseases has historically been a cornerstone of infection control. Early practices, such as septic wards and specialized hospitals, aimed to prevent cross-contamination during periods of limited treatment options. Today, isolation guidelines primarily exist for infections caused by highly transmissible or multidrug-resistant pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA), while the evidence for the strict separation of other infections is less clear. Particularly in light of resource limitations, staffing shortages, and the evolving understanding of infection control, this scoping review aims to evaluate the current evidence for isolating patients with post-operative infections in contemporary clinical settings.

This review was conducted using the Preferred Reporting Items for Systematic reviews and Meta-analysis Extension for Scoping Reviews (PRISMA-ScR) and employed the JBI PCC framework (Population: postoperative infection patients, Concept: isolation policies, Context: hospital wards). A comprehensive search was developed in collaboration with KU Leuven Library and conducted in Embase, Scopus, Web of Science, Cochrane (CENTRAL & CDSR), CINAHL, and ClinicalTrials.gov. Articles were screened independently by two researchers, using the online software Rayyan.

Of 10,061 **Abstracts** identified through the search strategy, 115 were selected for full-text screening. Of these, 14 articles met the final inclusion criteria for this review. Nine articles focused on patient isolation practices within surgical wards, while the remaining five articles focused on isolation practices in general hospital settings.

Of the included studies, nine focused on MRSA, two on extended spectrum beta-lactamase (ESBL)-producing pathogens, and one on Vancomycin-resistant *Enterococcus* (VRE). Isolation measures varied, including private rooms and cohort units, often combined with interventions like (nasal) screening, decolonization, enhanced hand hygiene, and targeted antibiotic prophylaxis.

The findings show that nearly all studies focus on resistant pathogens, underscoring the lack of evidence for routinely isolating post-operative infections. While a pathogen-specific isolation policy may be beneficial, no clear consensus can be drawn, as not all studies

demonstrate a reduction in infection incidence. Moreover, the absence of studies on non-resistant pathogens raises questions about the applicability of these results to broader patient populations. Given these limitations, combined with resource constraints, staffing shortages, and the evolving understanding of infection control, the routine use of septic wards appears outdated and is not recommended.

Po5_1.08

Polyacrylamide hydrogel for knee osteoarthritis: 5-year results from a prospective study

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Abstract

Introduction. Polyacrylamide hydrogel (iPAAG), is CE marked for treating symptomatic knee osteoarthritis (OA), meeting the need for an effective, long-lasting, and safe non-surgical option. This study evaluates the efficacy and safety of a single 6 ml intra-articular injection of iPAAG in participants with moderate to severe knee OA over a 5-year post-treatment period.

Method. This prospective multicentre study (3 sites in Denmark) involved 49 participants (31 females) with an average age of 70 (range 44 – 86 years). They received a single 6 mL iPAAG injection. All participants provided informed consent and re-consented to continue after 1 year. The study followed GCP principles and was approved by Danish health authorities and local Health Research Ethics committees. One of the sites closed after the first year, while twenty-seven participants from the other sites completed the 5-year follow-up.

The study evaluated WOMAC pain, stiffness, function, and Patient Global Assessment (PGA) of disease impact. Changes from baseline were analysed using a mixed model for repeated measurement (MMRM). Sensitivity analyses were applied on the extension data, where the MMRM analysis was repeated only including patients in the extension phase and an ANCOVA model was used, replacing missing values at 5-years with baseline values (BOCF).

Results. The originally planned MMRM analysis including all available data from the 49 treated participants showed clinically relevant and statistically significant decreases from baseline to 5 years for each of the 3 WOMAC subscale scores and the PGA (**Table 1**).

Table 1: Analyses of change from baseline to 5 years in transformed (0-100) WOMAC subscales and PGA

	Number of participants		LSMean (95% CI)	p-value
	At baseline	At 5 years		
WOMAC pain subscale				
Planned analysis	49	27	-14.6 (-21.4; -7.7)	0.0002
Extension participants	35	27	-15.6 (-22.3; -8.9)	<0.0001
Baseline carried forward	49	49	-9.1 (-14.1; -4.1)	0.0006
WOMAC stiffness subscale	49	27	-19.6 (-29.9; -9.3)	0.0006
WOMAC Phys. Function subscale	49	27	-12.5 (-19.8; -5.2)	0.0015
Patient Global Assessment	49	27	-13.4 (-23.3; -3.5)	0.0100

CI: confidence interval; LSMean: Least squares mean; PGA: Patient Global Assessment; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index

The analysis only using the data from the extension phase participants showed a similar change from baseline in the WOMAC pain subscale compared to the result of the planned analysis (**Table 1**). The BOCF analysis also showed a clinically relevant and statistically significant decrease in the WOMAC pain subscale from baseline. Seven new adverse events were reported between the 4-year and 5-year visits, with two reported as serious (acute myocardial infarction and femoral neck fracture). None of these seven events were related to the treatment.

Conclusions. This study shows that single injections of 6 ml intra-articular iPAAG were well tolerated and continued to provide clinically important effectiveness at 5-years after treatment.

Acknowledgements. The study was sponsored by Contura International A/S.

Po5_1.09

Rehabilitation And Functional Outcomes In Oncological Patients Treated With Megaprosthesis Reconstruction: A Systematic Review

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Abstract

Background. In recent years, there has been an increase in the number of performed megaprosthesis reconstructions following bone tumour resections, facilitating improved patient outcomes and improved postoperative quality of life. Despite this, no clear postoperative rehabilitative guidelines exist. The aim of this systematic review was to assess available rehabilitation protocols following megaprosthesis surgery and the associated functional outcomes.

Study Design & Methods. A systematic search was conducted in MEDLINE and EMBASE databases according to the updated Implementing Prisma in Exercise, Rehabilitation, Sport medicine and Sports science (PERSiST) guidelines. Studies describing rehabilitation protocols and functional outcomes following bone tumour resection and modular oncologic megaprosthesis reconstruction were included. All papers were individually assessed for methodological quality using the Joanna Briggs Institute (JBI) critical appraisal tool.

Results. The literature search generated 109 records, 25 were eligible for full text review and in total 11 studies were included in the final analysis. In total there were 308 patients with a mean age of 52.75 (S.D. 20.45). The mean patient follow-up period with functional assessment was 31.44 months. Rehabilitation protocols were assessed in 5/11 studies following proximal humerus resections with a mean dislocation rate of 14.5% (range 10-20%). All protocols advised brace immobilization for a period ranging between 10 days to 6 months. Superior Constant shoulder score was reported in patients with early active isometric exercises at 6 weeks. Only 1/11 studies assessed distal humerus resections, the time point of active arm mobilisation was dependent on prosthesis design. Proximal femur prosthesis rehabilitation protocol and functional outcomes were discussed in 5/11 studies, with mean reported dislocation rate of 12% (range 4-25%). Lower VAS scores were reported with protocols advising early mobilisation with independent weight bearing at 2 months. Two studies (2/11) assessed distal femur prosthesis, both studies reported similar protocols with full weight bearing 3 weeks following surgery. The methodological quality of the studies varied, 6/11 studies met at least 6 of the 10 evaluation criteria on the JBI appraisal tool and a further 2/11 studies met at least 50% of criteria.

Conclusions. There is much heterogeneity in protocols as well as measure outcomes. However, the results overall suggest that early mobilisation does not increase the risk of prosthesis dislocations and increases functional outcomes. Future designs of rehabilitation protocols should incorporate early active mobilisation of both upper and lower limbs following surgery and early full weight bearing following lower limb megaprosthesis reconstructions.

Po5_1.10

Evaluation of the Clinical Robotic-Assisted Total Hip Arthroplasty: A Meta-Meta-Analysis of Systematic Reviews and Meta-Analyses

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Abstract

Background: Total hip arthroplasty (THA) ranks among the most commonly performed orthopedic surgeries worldwide, mainly used to treat severe hip osteoarthritis. Recently, robotic-assisted THA (RA-THA) has emerged as a promising alternative to traditional manual procedures, potentially offering greater accuracy in implant placement and reducing intraoperative variability. However, the clinical benefits of RA-THA regarding functional outcomes, complication rates, and prosthesis longevity remain a matter of debate.

Objective: This study aims to systematically evaluate and synthesize existing systematic reviews and meta-analyses that compare RA-THA with conventional THA. The focus is on assessing methodological consistency, identifying potential biases, and examining the variability in reported clinical outcomes.

Methods: Following PRISMA guidelines, a meta-meta-epidemiological approach was employed. Comprehensive searches were conducted in databases such as PubMed, Web of Science, Embase, and Scopus for SRL-MA studies published from January 2014 to the present. The inclusion criteria encompassed systematic reviews with meta-analyses comparing RA-THA and conventional THA, particularly those focusing on clinical outcomes, implant accuracy, complication rates, and patient-reported results. Two independent reviewers performed data extraction and quality assessment using the AMSTAR-2 tool.

Results: A total of eight systematic reviews and meta-analyses were included, covering data on clinical outcomes, radiological accuracy, complication rates, and revision statistics. Compared to conventional THA, RA-THA demonstrated significantly improved implant positioning accuracy, especially concerning acetabular cup alignment and limb length discrepancy. However, no consistent evidence indicated better functional outcomes or reduced revision rates. Complication rates were comparable between the two techniques. The analysis also highlighted methodological issues, including heterogeneity and inconsistent outcome reporting.

Conclusions: While RA-THA consistently shows enhanced radiographic accuracy, its clinical superiority over conventional THA remains unproven. Challenges such as a prolonged learning curve, increased operative time, and high costs limit its adoption, particularly in resource-limited settings. To better understand the true clinical value of robotic assistance in hip arthroplasty, further high-quality randomized trials with long-term follow-up are essential.

Po5_1.11

Mechanical failure after primary internal fixation of unstable ankle fractures – a retrospective observational study

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Abstract

OBJECTIVES: The primary objective of this study was to observe and categorise patterns of mechanical failure following primary ankle internal fixation.

METHODS: Design: Retrospective observational study

Setting: Single Major Trauma Centre

Patient Selection criteria: A retrospective review was conducted, analysing electronic patient records (EPR) and radiographs of 897 patients who underwent internal fixation for unstable ankle fractures over five years (June 2014 to August 2018) at a university teaching hospital. Data on patient demographics, injury characteristics, surgical techniques, and complications were collected. For the sub-group of patients with mechanical failure, fracture patterns were further classified using AO/OTA, Lauge-Hansen, and Weber classifications (**Table 1**).

Outcome Measures and Comparisons:

Mechanical failures were categorised into three subtypes based on the direction of talar displacement and syndesmotic involvement (**Figure 1**): Type 1 (lateral talar instability with intact syndesmosis), Type 2 (lateral talar instability with disrupted syndesmosis), and Type 3 (posterior talar instability).

The inter-rater reliability of this classification system was evaluated using intraclass correlation coefficient (ICC) analysis.

Table 1. Fracture patterns for the study sample of patients with mechanical failure after primary ankle fixation (n=41)

	Failure subtypes			Total	Percentage/%
	Type 1	Type 2	Type 3		
Total	9	27	5	41	
Percentage/%	22%	66%	12%		100%
Fracture classifications					
AO/OTA:					
44A	0	0	0	0	0
44B1	0	0	0	0	0
44B2	4	5	0	9	22%
44B3	4	14	4	22	54%
44C1	0	2	0	2	5%
44C2	1	5	1	7	17%
44C3	0	1	0	1	2%
Lauge-Hansen:					
Pronation-Abduction (PAB)	0	4	0	4	10%
Pronation-External Rotation (PER)	1	6	1	8	20%
Supination-External Rotation (SER)	8	17	4	29	70%
Supination-Adduction (SAD)	0	0	0	0	0
Weber:					
Weber A	0	0	0	0	0
Weber B	8	19	4	31	76%
Weber C	1	8	1	10	24%

RESULTS: The revision rate after primary ankle internal fixation was 6.6% (59/897), with mechanical failures accounting for 69% (41/59) and infective failures for 31% (18/59). Patients in whom the primary ankle fixation failed were significantly older than the group in whom the primary ankle fixation did not fail (mean age 60 vs. 52 years, $p=0.0018$). The mean ICC values for the classification system was 0.84, indicating good inter-rater reliability.

CONCLUSIONS: The revision rate for unstable ankle fractures treated with primary internal fixation was 6.6%, with the majority of revisions attributed to mechanical failures. Lateral talar instability, particularly when associated with syndesmotic disruption, was the most common cause of mechanical failure. Our grading system demonstrated high inter-rater reliability and provides a tool for categorising these failures.

Figure 1 Examples of three patterns of failures identified (Types 1/2/3)



Po5_1.15

Safety and Accuracy of Percutaneous Osteotomy and Intramedullary Nailing Compared to Open Osteotomy with Plate Fixation in Femoral Derotational Surgery – a randomized controlled trial"

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Abstract

Increased femoral anteversion (IFA) is a common developmental condition in children, with approximately 80% resolving spontaneously. However, when IFA persists into adolescence and adulthood, it can lead to functional issues such as tripping, as well as hip and knee pain. For symptomatic cases, femoral derotational osteotomy remains the gold standard treatment. Traditionally, open osteotomy with plate fixation has been the preferred approach. Minimally invasive techniques, such as percutaneous osteotomy with intramedullary nailing, offer potential advantages, but reports on their use are limited, particularly regarding precision. This study compares the safety, accuracy, and clinical outcomes of these two surgical techniques.

Methods: This single-center randomized controlled trial included 30 patients (49 rotational osteotomies; mean age 13.6 ± 1.6 years) with a CT-verified anteversion angle of $\geq 30^\circ$. Patients were randomized to either percutaneous osteotomy with intramedullary nailing ($n=26$ hips) or open osteotomy with plate fixation ($n=23$ hips). Primary outcomes assessed the safety of the procedures and the accuracy of derotation. Secondary outcomes included functional scores (Barnhöft, KOOS-Child), operative metrics, recovery, radiographic outcomes, and patient satisfaction.

Results: Both techniques achieved significant anteversion correction (mean preoperative AV: $51.4^\circ \pm 9.6^\circ$ mean correction: $29.5^\circ \pm 8.7^\circ$). Deviation from intended correction was minimal (nail: $6.3^\circ \pm 3.9^\circ$; plate: $7.0^\circ \pm 5.9^\circ$; $p=0.586$), with 88.5% of nail and 74.2% of plate procedures achieving $<10^\circ$ deviation. Healing of the osteotomies occurred within 3 to 6 months, and there were no non-unions, with low reoperation rates (nail: 3.9%; plate: 4.4%). Both groups showed significant improvements in patient-reported outcome measures (PROMs), with no significant differences in KOOS-Child subdomains or satisfaction scores.

Discussion: The results demonstrate that both techniques are effective for femoral derotational osteotomy, achieving similar safety, accuracy, and functional outcomes. The intramedullary nailing approach offers the added benefit of being less invasive, making it a suitable option for selected patients. Complication rates, including reoperations and deviation from intended correction, were comparable between groups. However, surgeon expertise and individual patient anatomy should guide the choice of technique.

Conclusion: Percutaneous osteotomy with intramedullary nailing is a safe and effective alternative to open osteotomy with plate fixation for femoral derotational surgery. Both techniques significantly improve quality of life and functional outcomes. Future research with larger cohorts and longer follow-up is needed to further refine these findings.

Po5_1.16

Inverse relationship between femoral lateralization and neck-shaft angle is a joint event after intramedullary nailing of per trochanteric fractures

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Abstract

This study explored the relationship between femoral lateralization and femoral neck-shaft angle after intramedullary nail (IM) fixation for per trochanteric fractures. 70 patients (AO/OTA 31A1-2) were investigated. Anteroposterior (AP) and lateral X-ray views pre- and post-operation were recorded. Patients were classified into three groups according to the position of the medial cortex of the head-neck fragment to that of the femoral shaft: being slightly superomedial (positive medial cortex support, PMCS), being smoothly contacted (neutral position, NP) or being displaced laterally (negative medial cortex support, NMCS) (Fig.1). Patient demographics, femoral lateralization, and neck-shaft angle were measured and statistically analyzed pre- and post-operation. Functional recovery was evaluated by Harris score 3- and 6- months post-operation. All cases ultimately demonstrated radiographic fracture union. There was a tendency to have an increased neck-shaft angle (valgus alignment) in the PMCS group and increased femoral lateralization in the NP group ($p < 0.05$). Among those three groups, the change in femoral lateralization and neck-shaft angle was statistically different ($p < 0.05$). An inverse relationship between femoral lateralization and femoral neck-shaft angle was observed (Fig.2). Femoral lateralization increased correspondingly when the neck-shaft angle continuously decreased from the PMCS group to the NP group and then to the NMCS group, and patients in the PMCS group had better

functional recovery than the other two groups ($p < 0.05$). Femoral lateralization was commonly produced after IM fixation for per trochanteric fractures. The fracture fixed in PMCS mode possesses the slightest change in femoral lateralization while maintaining valgus alignment of the femoral neck-shaft angle and good functional outcome, which is superior to NP or NMCS mode.

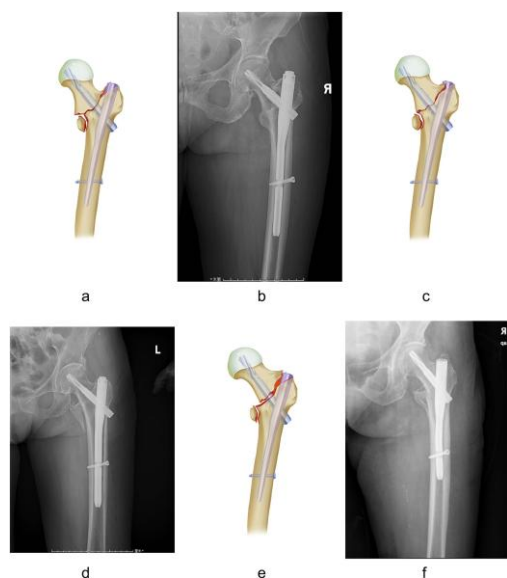


Fig.1. Various medial cortical support effects demonstrated as schematic diagram (a: PMCS, c: NP, e: NMCS) and X-ray view (b: PMCS, d: NP, f: NMCS).

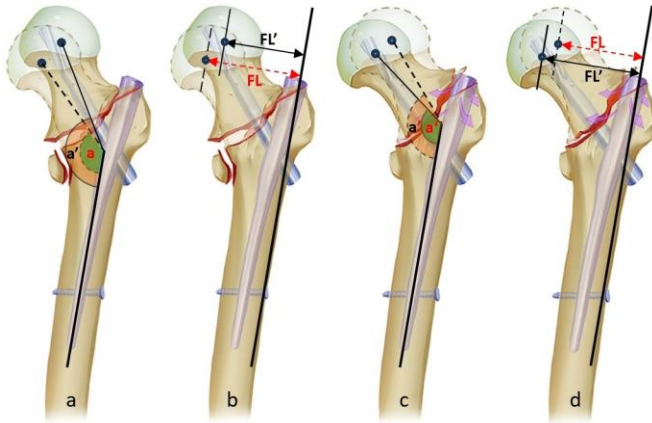


Fig.2. Underlying mechanism of inverse relationship between neck-shaft angle and femoral lateralization. a-b : increased neck-shaft angle (a) with decreased femoral lateralization(b); c-d: decreased neck-shaft angle (c) with increased femoral lateralization (d); Normal anatomy of femoral head and neck is shown with dashed outline. a: normal neck-shaft angle; a': neck-shaft angle after IM insertion; FL: normal femoral lateralization; FL': femoral lateralization after IM insertion;

Po5_1.18

Mid-Term Results of The Modified Bereiter Trochleoplasty for patients with recurrent patellofemoral instability due to severe trochlear dysplasia: The Oswestry Experience

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Abstract

Purpose. Trochleoplasty is an effective surgical procedure for patients with recurrent patellofemoral instability attributed to severe trochlear dysplasia. We have previously published our short-term outcomes, with good to excellent results in knee function and quality of life as measured by IKDC, Kujala and EQ-5D scores, and with an acceptable complication profile when compared to the available literature. The aim of this study is to report on our mid-term outcomes and identify any post-operative surgical sequelae which may only manifest during mid to long-term follow-up.

Methods. A longitudinal patellofemoral database was checked to identify all trochleoplasties performed from inception. Patients with less than 3 years follow-up data were excluded from our final analysis. Post-operative IKDC, Kujala and EQ-5D results were compared to baseline pre-operative scores. Due to the non-parametric nature of the data, median and interquartile range (IQR) were determined for pre-operative and mid-term follow-up scores.

Results. A total of 52 trochleoplasties were performed between 2013-2019; with 28 trochleoplasties with > 3 years follow-up data available for analysis. The median time from surgery to follow-up was 5.3 yrs (IQR 3.9 – 7.0 years). The median Kujala score improved from 57.0 (IQR 45 – 66) to 96.3 (IQR 83 -100). Median IKDC score improved from 42.6 (IQR 35.9 – 51.3) to 92.6 (IQR 71.6 – 98.7). Median EQ-5D scores also improved from 0.691 (IQR 0.414 – 0.727) to 1.000 (IQR 0.8178 – 1.000). Improvement in Kujala, IKDC and EQ-5D scores were all statistically significant ($p < 0.001$) in all domains. There were no further symptoms of patella instability, or complications related to chondral necrosis or patellofemoral osteoarthritis.

Conclusion. The Modified Bereiter trochleoplasty remains a safe and effective procedure in addressing severe trochlear dysplasia and in treating recurrent patellofemoral instability, with sustained improvement in knee function and quality of life at a mean follow-up of 5.3 yrs.

Po5_1.19

Incidence and Diagnostic Modalities used to detect Soft Tissue Knee injuries associated with fractures of the tibial plateau: A systematic review.

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Abstract

Purpose. Damage to the soft tissue structures of the knee following tibial plateau fractures, appear to be critical in determining the incidence of post-traumatic arthritis and the need for further surgery. Nevertheless, due to the nature of the injury and the relative paucity of the subcutaneous tissues about the knee, detecting such injuries remains a challenge, with various modalities used to confirm, each with several limitations. Therefore, the aim of this systematic review is to determine and evaluate the incidence and the different diagnostic modalities used for assessing the soft tissue injuries of the knee when a proximal tibial plateau fracture is sustained.

Methods. A systematic search of MEDLINE 1950 – 2023, EMBASE 1980 – 2023 and AMED (1985 – 2023) was performed in accordance with the PRISMA guidelines to identify studies written in English language, which looked at and compared the incidence and the use of different modalities to detect soft tissue injuries following a tibial plateau fracture.

Results: 11 studies met the inclusion encompassing a total of 468 patients with an incidence ranging from 42% to 100%. The overall mean incidence of an ACL injury is 26% (n=122, range 10% - 49%), a PCL injury 32% (n=149, range 0% - 74%). The collateral ligaments appear to be affected similarly with a mean incidence of 15% for a medial collateral ligament (MCL) injury and 17% for a lateral collateral ligament (LCL) injury. The 2% difference between the two ligaments may not be generalizable due to confounding factors including the type of the fracture sustained and the mechanism of injury. The overall mean incidence of a lateral meniscus (LM) injury including a complete tear or avulsion is 50% (n=232, range 5% - 91%) as compared to the medial meniscus (MM) 30% (n=141, range 13% - 55%). The use of MRI appeared to be advantageous but the timing of the scan following the injury appears to be heterogeneous in the literature. MRI was associated with several false positive and false negative results.

Conclusion. Soft tissue injuries associated with tibial plateau fractures remain a challenge to the orthopaedic surgeon in diagnosis and treatment. From the available literature, the use of MRI in the surgical planning of tibial plateau fractures is recommended. However, the findings should be interpreted with caution, as false-negative and false-positive results could occur.

Po5_1.23

Ventilation Plaster As a Unique Way to Overcome Cutaneous Problems Associated During and Post Plaster Removal

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Abstract

Introduction: Plaster immobilization is the conventional procedure for fracture management¹. However, it is known that plaster hardening which is an exothermic, heat generating reaction causes burns and blisters further leading to secondary bacterial infections of the skin and eventually scarring. Prolonged plaster immobilization could also compromise the skin surface with impurities getting trapped creating localized abrasion and scarring. We hypothesized that a ventilation plaster consisting of holes made at a certain distance from each other would help in preventing these skin conditions²⁻⁴.

Method: We performed a clinical study of 200 adult patients divided equally in two groups with limb immobilization in synthetic plaster, without any prior medical condition, skin condition or allergy to the plaster material. With informed medical consent, the study was conducted from March 2022 to January 2023. The patients were divided into two groups, one with conventional limb immobilized in plaster and the other group with ventilation plaster consisting of holes ½ inch in diameter and 5-7 cm apart. We studied the patients on parameters like sweating, itching, discomfort and cast claustrophobia while the plaster was immobilized. Post-plaster removal, we assessed the two groups on conditions like dry skin, rashes, blisters, sores, exfoliation, xerosis, pigmentation, hypertrichosis, scarring, and atrophy.

Results: We observed that 55 patients with normal plaster experienced itching during plaster as compared to 14 patients with ventilation plaster which was significant. 89 patients with normal plaster had xerosis post plaster removal compared to 53 with ventilation plaster. Pigmentation was seen in 45 patients with normal plaster as opposed to 26 in ventilated plaster. In hypertrichosis, atrophy and scarring, the numbers were comparable.

Conclusion: Our study is a one of its kind study which improves upon the traditional method of limb



immobilization. Ventilating plaster is a new concept which solves the existing plaster associated cutaneous problems in patients and provides better rehabilitation. We suggest using ventilation plaster to prevent discomfort, rashes and other skin allergies during and after limb immobilization.

holes 5-7 cm apart

Figure: A patient with Ventilation plaster having

Po5_1.24

Data Insights on the Risks of Local Heat and Massage in Gouty Arthritis Treatment

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Abstract

Introduction: Gouty arthritis is a common inflammatory condition caused by the deposition of uric acid crystals in the joints, leading to intense pain, swelling, and functional impairment. Thermal therapies, including hot and cold fomentation, are often used as adjunctive treatments for managing inflammation and pain in various musculoskeletal conditions. However, the efficacy of these therapies in acute gout remains underexplored. This study aims to compare the effects of hot fomentation versus cold fomentation in patients experiencing acute gout flares, assessing pain reduction, swelling, joint mobility, and overall patient satisfaction.

Case Report: A randomized controlled trial (RCT) was conducted with 2,400 patients diagnosed with acute gouty arthritis, divided into two equal groups: Group A received hot fomentation (38°C–42°C) and Group B received cold fomentation (5°C–10°C). Both interventions were applied twice daily for 20 minutes over 5 days, alongside standard gout care. Pain intensity was measured using the Visual Analog Scale (VAS), while joint circumference, range of motion, and patient satisfaction were recorded at baseline, day 3, and day 5. Statistical analyses compared outcomes between the two groups.

Results: Cold fomentation significantly reduced pain intensity (VAS score reduction of 68% by Day 5) compared to hot fomentation (26% reduction, $p < 0.001$). Joint swelling decreased by 25% in the cold group versus 5% in the hot group, with cold therapy also leading to greater improvements in joint mobility (average increase of 15 degrees versus 5 degrees, $p < 0.01$). Notably, 35% of patients in the hot fomentation group experienced flare-ups, compared to only 2% in the cold fomentation group ($p < 0.001$). Patient satisfaction was also higher in the cold group, with 85% of participants reporting satisfaction versus 30% in the hot group.

Conclusion: This study demonstrates that cold fomentation is significantly more effective than hot fomentation in managing acute gouty arthritis. Cold therapy provided superior pain relief, reduced swelling, improved joint mobility, and minimized the risk of symptom exacerbation. In contrast, hot fomentation often worsened symptoms, making it unsuitable for acute gout management. These findings suggest that cold fomentation should be the preferred thermal therapy for gout flare-ups, while heat applications should be avoided.

Keywords: Gouty arthritis, hot fomentation, cold fomentation, thermal therapy, pain management, swelling, joint mobility, flare-ups, patient satisfaction.

Po5_1.25

Atypical Presentation of Gout: Idiopathic Retrocalcaneal Pain in 400 Patients

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Abstract

Introduction: Idiopathic retrocalcaneal pain is frequently linked to overuse injuries or inflammation around the Achilles tendon and surrounding bursae. However, systemic conditions such as gout, characterized by monosodium urate crystal deposition, can also present as retrocalcaneal pain. Gout commonly affects the first metatarsophalangeal joint, but atypical presentations like retrocalcaneal involvement are increasingly recognized. This study aims to assess the presentation, diagnosis, and treatment outcomes of gout in patients presenting with idiopathic retrocalcaneal pain.

Materials and Methods: This retrospective study analyzed clinical data from 400 patients diagnosed with gout and presenting with idiopathic retrocalcaneal pain between 2019 and 2023 at a Tertiary Care Centre. Diagnosis was based on clinical examination, serum uric acid levels, imaging, and in some cases, synovial fluid analysis. Patient demographics, diagnostic methods, and treatment outcomes, including pain (VAS) and functionality (FADI) scores, were evaluated.

Results: Of the 400 patients, 290 (72.5%) were male, and 110 (27.5%) were female, with a mean age of 56.8 ± 12.3 years. Pre-existing gout was present in 58% of patients, and serum uric acid levels were elevated in 75%. Synovial fluid analysis, performed in 65 patients, confirmed monosodium urate crystals in 92.3% of cases. Treatment resulted in significant reductions in pain (VAS: 7.8 ± 1.4 to 3.2 ± 1.1 , $p < 0.05$) and improved functionality (FADI: 58.4 ± 5.7 to 85.7 ± 6.3). Recurrence of symptoms occurred in 25% of patients, particularly in those who did not adhere to urate-lowering therapy.

Conclusion: This study emphasizes the importance of considering gout in the differential diagnosis of idiopathic retrocalcaneal pain. Early diagnosis and appropriate management can lead to favorable outcomes and lower recurrence rates. Clinicians should remain vigilant for atypical presentations of gout to prevent prolonged discomfort and functional impairment.

Keywords: idiopathic retrocalcaneal pain, gout, hyperuricemia, monosodium urate crystals, Achilles tendon, atypical gout, urate-lowering therapy.

Po5_1.26

Prevalence and Clinical Characteristics of Gout in Premenopausal and Postmenopausal Females: A Comparative Study

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Abstract

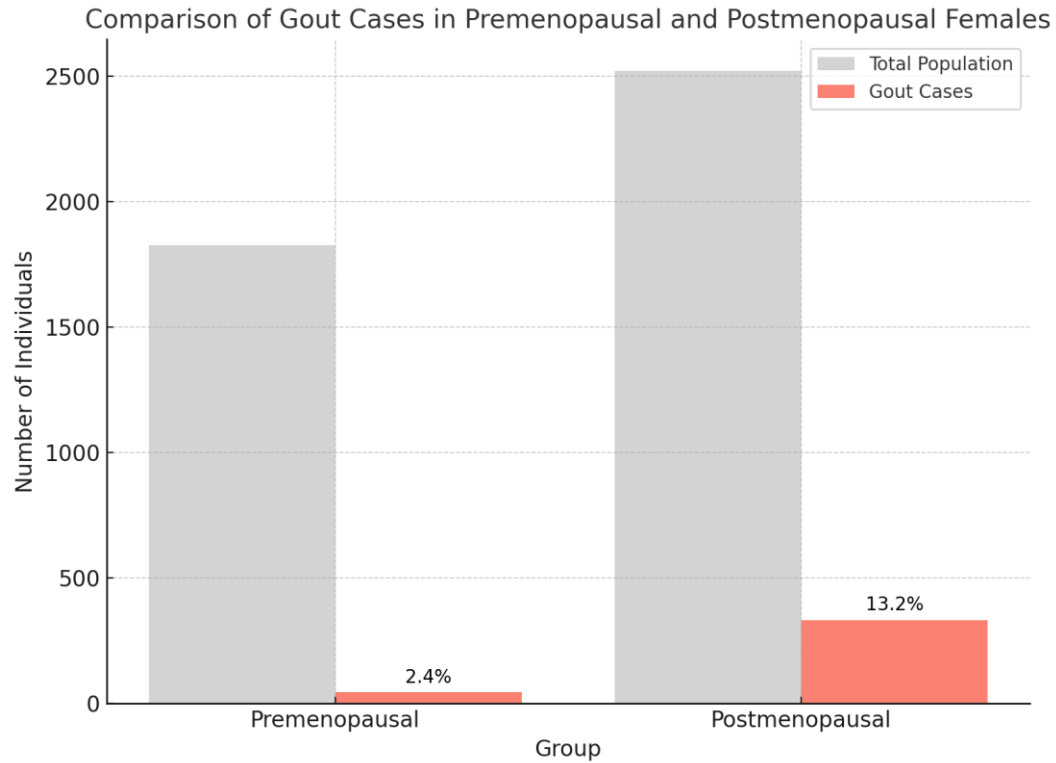
Introduction: Gout, an inflammatory arthritis caused by monosodium urate crystal deposition, is influenced by hormonal changes, particularly the protective uricosuric effects of estrogen. This study examines the prevalence, clinical characteristics, diagnostic methods, and treatment outcomes of gout among premenopausal and postmenopausal females, emphasizing the role of hormonal changes in disease manifestation.

Materials and Methods: A cross-sectional study was conducted involving 4,350 females, comprising 1,827 premenopausal and 2,523 postmenopausal participants. Gout diagnosis was based on clinical symptoms, including acute joint pain, erythema, and swelling, confirmed through elevated serum uric acid levels (>6 mg/dL). Symptom onset, affected joints, and treatment outcomes were documented. Management included dietary modifications, nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, and corticosteroids, adjusted for safety and efficacy.

Results: Gout prevalence was 2.4% (n=44) in premenopausal females and 13.2% (n=333) in postmenopausal females. The most commonly affected joint was the first metatarsophalangeal joint (56%), followed by the knees (23%) and ankles (15%). Average symptom onset occurred at 35 years in premenopausal females and 54 years in postmenopausal females. Elevated serum uric acid levels confirmed gout in all cases. Dietary adjustments and NSAIDs provided symptom relief in 89% of cases, while colchicine and corticosteroids were reserved for severe or refractory cases.

Conclusion: The study highlights a markedly higher prevalence of gout in postmenopausal females compared to premenopausal females, underscoring the protective role of estrogen against hyperuricemia. Accurate diagnosis and timely treatment, including lifestyle modifications and pharmacotherapy, are critical for effective gout management. These findings emphasize the need for targeted interventions and awareness of hormonal influences on gout.

Keywords: Gout, menopause, premenopause, hyperuricemia, hormonal influence, uric acid, joint pain, gout treatment, estrogen.



The figure shows the comparison of total population and gout cases in premenopausal and postmenopausal females. The percentages of gout cases within each group are annotated above the respective bars for clarity.

Po5_1.27

Investigating the effects of inflammatory factors on joint tissues using near-infrared (NIR) spectroscopy on explant culture medium

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Abstract

Introduction Chronic joint disorders, like osteoarthritis (OA), are degenerative diseases characterized by local inflammation and complex interactions within joint structures (1). Despite significant progress, many aspects of their pathophysiology remain unclear (2). The role of inflammatory biomarkers is not fully understood due to the lack of reliable models and real-time detection methods (3). Inflammatory explant degeneration models often examine glycosaminoglycan (GAG) and nitric oxide (NO) release (4). These biomarkers are assessed using methods like ELISA and mass spectrometry, which are accurate but costly and time-consuming (5). We aim to address these shortcomings by using near-infrared spectroscopy (NIRS) for rapid, non-destructive assessment of GAG and NO release, combined with machine learning (ML) models (6,7).

Methodology: Bovine osteochondral explants and synovium (n=24) were co-cultured in DMEM high glucose (HG) and low glucose (LG) media, supplemented with antibiotics, ITS + Premix, non-essential amino acids, and ascorbic acid, with or without TNF and IL1B. GAG and NO release were measured daily over 14 days (336 samples) using dimethyl-methylene blue and Griess assay, respectively. NIR spectral data were acquired in transfection mode using a fiber optic probe and spectrometer (900-1700nm, StellarNet, USA) and analyzed with the QUASAR-Orange tool. ML algorithms (Random Forest, Adaptive Boosting (AdaBoost), Support Vector Machine (SVM), Gradient Boosting) classified the spectral data into four classes based on medium composition.

Results: The production of GAG and NO, measured using assays, was significantly influenced by glucose concentration and inflammatory factors. High glucose and inflammatory factors produced the highest GAG levels. Inflammatory factors increased GAG and NO levels in both glucose conditions. High glucose alone increased GAG levels but did not significantly affect NO levels. Preliminary results show that NIRS (Figure-1a), combined with ML algorithms, can effectively classify the culture media based on their composition. These models showed high accuracy in distinguishing between different media conditions, enabling precise monitoring of GAG and NO levels (Figure-1b, Table-1).

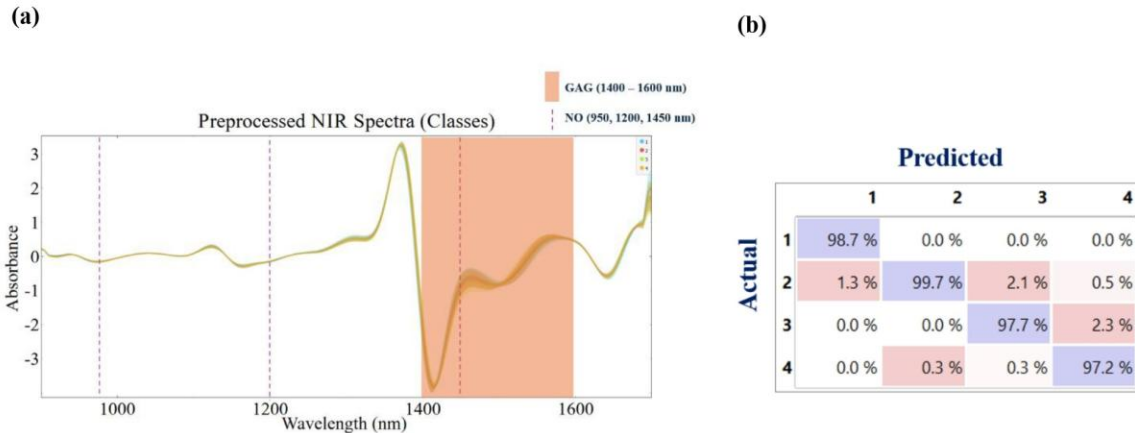


Figure 1 – Classification of NIR spectra based on culture media composition. (a) **Near Infrared (NIR) preprocessed spectra.** NIR spectral classification was performed based on glucose and inflammatory factors, with spectra ranging from 900 to 1700 nm. The baseline was corrected, and the spectra were smoothed using Gaussian smoothing ($SD=10$) and a Savitsky-Golay filter (window = 13, polynomial order = 3, derivative order = 2), then normalized to SNV. Changes in the spectra for GAG were correlated to the range of 1400 to 1600 nm. While there was no direct correlation for NO, peaks at 950, 1200, and 1450 nm were associated with changes in NO levels in the medium. (b) **Confusion matrices of ML model-** The confusion matrices show that the SVM model, the best-performing one, accurately identifies over 97% of samples in each class with minimal misclassifications (Class 1 - low glucose, Class 2 - low glucose with inflammatory factors, Class 3 - high glucose, and Class 4 - high glucose with inflammatory factors.).

Table 1 - Classification performance metrics for NIR spectra of medium samples across four classes: Class I - low glucose, Class II - low glucose with inflammatory factors, Class III - high glucose, and Class IV - high glucose with inflammatory factors. Metrics include AUC (Area Under the Curve), CA (Classification Accuracy), F1 (F1 Score), Precision, Recall, and MCC (Matthews Correlation Coefficient).

Model	AUC	CA	F1	Prec	Recall	MCC
Gradient Boosting	1.000	0.996	0.996	0.996	0.996	0.994
Random Forest	1.000	0.996	0.996	0.996	0.996	0.995
SVM	0.999	0.983	0.983	0.984	0.983	0.978
AdaBoost	0.936	0.904	0.904	0.904	0.904	0.872

Discussion: We demonstrate the potential of NIRS for assessing the influence of inflammatory factors on biomarker release in culture media. Preliminary results show NIRS can rapidly detect low concentrations of GAG and NO in biofluids, offering insight into changes in media composition. Ongoing studies aim to quantify GAG and NO concentrations using NIRS.

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Po5_1.29

Autonomic dysfunction with sympathetic dominance in patients with late-stage osteoarthritis recovered one year after total knee arthroplasty

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Abstract

INTRODUCTION: The degenerative and mild chronic inflammatory joint disease osteoarthritis (OA) results in limited mobility and chronic pain. Since the pathophysiology of OA is multifaceted and highly complex, it is still not entirely understood. We recently demonstrated that heart rate variability (HRV) is reduced in individuals with late-stage OA indicating an autonomic dysfunction with sympathetic dominance. The present study investigated whether autonomic balance is restored one year after total knee arthroplasty (TKA).

METHODS: The study cohort consisted of 76 patients with late-stage knee joint OA before and one year after TKA. HRV parameters were determined from electrocardiograms and markers that indicate parasympathetic (high frequency (HF) power, pNN50 (%), RMSSD (ms), and SDRR (ms)) and sympathetic (low frequency (LF) power) activities were evaluated. Perceived pain and perceived chronic stress levels were assessed using questionnaires (PSQ and WOMAC). In addition, ELISA was used to analyse the ratio of the stress hormones cortisol and dehydroepiandrosterone sulfate (DHEAS) as well as IL-6 and CRP concentrations in serum samples. Linear regression analysis was performed to account for clinical baseline characteristics such as age, sex, BMI and all other measured parameters as potential HRV confounders. Additional serum proteomics analysis was performed to identify further differences.

RESULTS: The time point of analysis (ToA) had a significant impact on all HRV parameters. The HRV parameters SDRR ($p < 0.001$) and RMSSD ($p = 0.037$) increased one year after TKA in comparison to the respective pre-TKA levels without any influence of the confounders. LF ($p < 0.001$) power increased, while LF/HF ($p = 0.02$) ratio decreased one year after TKA. The perceived level of chronic stress did not alter with TKA, however the pain level significantly decreased. The cortisol/DHEA-S ratio significantly decreased one year after TKA ($p = 0.048$), however, elevated IL-6 and CRP concentrations did not fall after TKA. Evaluating serum proteomics data is the focus of our current work.

DISCUSSION: One year after TKA, HRV measures in patients with late-stage OA demonstrated a decreased sympathetic activity. Thus, these patients seem to have partially

regained autonomic balance. This is supported by the decreased serum cortisol/DHEA-S ratio, although systemic mild inflammation did not decrease. In conclusion, the autonomic tone seems to be impacted in OA and might even play a role in OA pathogenesis. Therefore, the autonomic nervous system could represent a promising novel potential therapeutic target by strengthening the parasympathetic activity.

Po5_1.30

Does intramedullary nailing through open growth plates cause harm? A scoping review

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Abstract

Purpose: This scoping review aimed to map the literature on effects of growth plate violation with an intramedullary locking nail (IMN) at the knee in skeletally immature patients. It focused on assessing clinical and experimental outcomes, with particular attention to complications such as growth arrest and deformities.

Methods: This scoping review followed the PRISMA extension for Scoping Reviews. The protocol was registered at Open Science Framework. A comprehensive search of PubMed, Embase, Cochrane Library, Web of Science, and Scopus was performed for studies involving pediatric patients with open growth plates treated with IMNs at the knee, as well as animal studies assessing physeal violations.

Results: Ten studies were included, comprising six clinical studies, three animal studies, and one review. The clinical studies included 89 pediatric patients treated either with retrograde femoral or antegrade tibial nailing. The youngest patient was a 7-years old female, and there were no reported cases of growth arrest following IMN. Animal studies demonstrated that up to 7% of physeal violation did not result in significant growth disturbances, but exceeding this threshold led to growth inhibition. Additionally, implant removal showed mixed results with early bone bridge formation noted in few cases.

Conclusions: This review indicates that limited physeal violations by IMN may not cause significant growth disturbances, while exceeding certain thresholds could result in adverse outcomes. The current evidence is insufficient to make recommendations on the use of IMN nails at the knee in skeletally immature patients, particularly to make recommendations regarding age, femur/tibia and sex. Further research is needed to establish long-term outcomes and optimal guidelines for safe physeal instrumentation.

Objective

Map the literature and highlight gaps in knowledge on growth plate violation in pediatric patients treated with rigid retrograde femoral or antegrade tibial IMN entering through the knee



Methods

5 databases



3976 unique citations

10 studies included

- 6 clinical
- 3 animal
- 1 review



Conclusion

Clinical studies:
No cases of growth arrest

Animal studies:
physeal violation of less than 7% did not result in significant growth disturbances



Po5_1.32

Geometric Modeling of Forearm Rotation for Subject-Specific Clinical Applications

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Abstract

Introduction: Existing forearm rotation models usually require multiple CT scans, sophisticated computations or do not satisfy joint constraints. The proposed model is a geometric model that requires a single CT scan, minimizing radiation exposure. It incorporates joint constraints without considering forces and uses bone morphology without explicitly modeling soft tissues. Compared to existing models that are more suitable for medical research, it is an approach that has the potential to be more easily applied to routine surgery planning.

Methods: As a start, the model considers only the distal radioulnar joint (DRUJ) constraint, which is defined as the joint gap size (gap constraint), preservation of gap size across the entire contact surface (pose constraint) and the planar relationship between DRUJ landmarks (plane constraint). The model rotates the radius about an axis, defined by manually annotated rotation centers according to known estimations, and adjusts the ulna to meet DRUJ constraints. It is applied to generate bone poses for 10 cadaveric forearms.

Results: The gap size and plane constraints are fully satisfied. The pose constraint, evaluated through visual inspection of 5 representative poses, is fully satisfied for 3 subjects (Fig. 1), partially satisfied for 6 subjects and not satisfied for 1 subject.

Conclusion: The model can satisfy the pose constraint for the majority of subjects. Its ability to satisfy the pose constraint is sensitive to rotation center placement and non-concentricity of the DRUJ contact surfaces. Continuing work will focus on effecting forearm rotation without a known axis and fully satisfying constraints at all major joints.

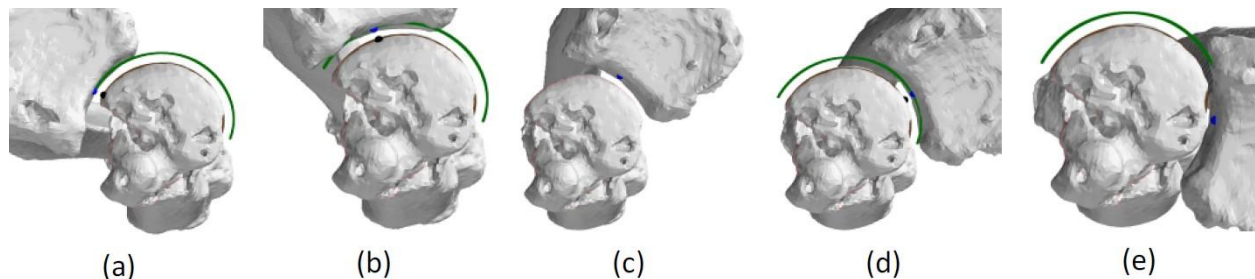


Figure 1: Bone poses generated by proposed model for a subject that fully satisfies gap size, pose and plane constraints. (a) Supinated pose. (c) Initial pose. (b, d) Intermediate poses. (e) Pronated pose.

Po5_1.33

"FengChao" Titanium Plate for Acromioclavicular and Coracoclavicular Ligament Reconstruction in the Treatment of Acromioclavicular Joint Dislocation

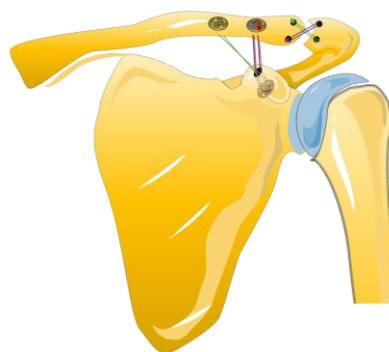
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Abstract

[Objective] To introduce the surgical technique and preliminary clinical outcomes of using a "Fengchao" titanium plate for acromioclavicular (AC) and coracoclavicular (CC) ligament reconstruction in the treatment of acromioclavicular joint dislocation.

[Methods] From January 2019 to January 2021, a "Fengchao" titanium plate was used for AC and CC ligament reconstruction in 20 patients with acromioclavicular joint dislocation. A horizontal incision of approximately 7 cm was made above the coracoid process to expose the base of the coracoid



and the superior surface of the acromioclavicular joint. Kirschner wires were used to create bone tunnels in the coracoid, clavicle, and acromion. The honeycomb titanium plate and ultra-strong sutures were introduced into the bone tunnels through a guide wire, and the acromioclavicular joint was reduced. The sutures were tightened with sliding knots to complete the fixation. [Results] All patients were followed up, with an average follow-up time of (13.25 ± 1.16) months. Compared with preoperative values, the VAS scores $[(5.0 \pm 1.1), (1.5 \pm 0.5), P < 0.001]$, Constant-Murley scores $[(56.6 \pm 3.8), (89.6 \pm 3.6), P < 0.001]$, and ASES scores $[(63.4 \pm 5.0), (90.5 \pm 3.8), P < 0.001]$ were all significantly improved at the final follow-up. Compared with preoperative values, the coracoclavicular distance (CCD) $[(23.6 \pm 3.5) \text{ mm}, (10.2 \pm 1.4), P < 0.001]$ was significantly improved at the final follow-up. [Conclusion] The use of "Fengchao" titanium plate for AC and CC ligament reconstruction in the treatment of acromioclavicular joint dislocation provides good fixation strength, a low incidence of postoperative complications, and is conducive to the recovery of shoulder joint function.



Po5_1.37

Impact of short tibial length on the development of knee osteoarthritis

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Abstract

Background: Osteoarthritis of the knee is the leading cause of disability worldwide. Previous studies showed association of bone diameter and bone length discrepancy as risk factors of knee osteoarthritis. However, few studies examine whether segmental discrepancy in the bones of lower limb have any association with knee osteoarthritis.

Objective: The purpose of this study was to investigate the effects of short tibia on the development of knee osteoarthritis.

Methods: Participants >50 years of age who were treated with various knee complain were enrolled. Full length Anteroposterior view x-rays were used to assess the length of mechanical axis of tibia, femur and the anatomical axis of femur. Kellgren-Lawrence scale was used for selecting the case and control group. The two groups were compared based on the length of tibia, femur and on tibial femoral length ratio.

Results: N=692 KL grade >2 were enrolled in case and N=369 KL<2 in control. The average age was 67.35±6.6 and 66.49±4.7 years in case and control group, F=1.277 and P = 0.0519. The average tibia mechanical axis length was 337.2±26.50 mm in case and 346.1±24.72mm in control, F=1.155 and P<0.0001. The mechanical axis of femur mean was 419.5±29.9mm in case and 419.2±30mm in control F=1.009 and P=0.234, the femur anatomical axis mean was 416.4±29.85mm in case and 413.3±29.48mm in control F=1.022 and P=0.1136. The ratio of Tibia mechanical axis to Femur mechanical axis was 0.802±0.030mm in case and 0.829±0.027mm in control F=1.312, P value < 0.0001. And the ratio of tibial mechanical axis with that of femur anatomical axis was 0.808±0.03mm in case and 0.836±0.027mm in control with F= 1.272, P value <0.0001.

Conclusion: This is to our knowledge, based on the findings of the present study the short tibial length relative to femur mechanical and anatomical length found to have significant impact to the development and progression of ipsilateral knee osteoarthritis.

Po5_1.40

The effect of femoral positioning deviation from the Schöttle point on graft tension in medial patellofemoral ligament reconstruction

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Abstract

The medial patellofemoral ligament (MPFL) is one of the primary stabilizing structures of the patella. The isometricity of the graft is critical for patellar stability and knee joint function after MPFL reconstruction. Femoral attachment of MPFL significantly affects the isometricity of the graft. This study aimed to investigate the impact of deviations from the anatomical femoral position on graft tension variations at different knee flexion angles. Three fresh-frozen human cadaveric knee specimens were used in this study. Graft tension was measured under different femoral attachment conditions at knee flexion angles of 0°, 30°, 60°, 90°, and 120°. The results revealed that, except for the position 1 cm posterior to the Schöttle point, all other deviations from the anatomical position exhibited higher tension variability coefficients compared to the Schöttle point. This indicated that deviations from the Schöttle point lead to greater variations in graft length and tension during knee joint motion, potentially affecting postoperative patellar tracking and knee joint function. This study highlighted the critical importance of precise femoral attachment positioning in MPFL reconstruction to ensure patellar stability and optimal functional recovery post-surgery.

Po5_1.41

Comparison of hip abductor muscle composition and its age-related alterations between the affected and the contralateral hip joints in female patients with unilateral osteoarthritis of the hip

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Abstract

Introduction: Fatty infiltration in the hip abductors is found in hip osteoarthritis (OA). Currently, it remains uncertain whether age is associated with the hip abductor muscle composition in unilateral hip OA. This study aimed to compare hip abductor muscle composition and its age-related alterations between the affected and the contralateral hip joints in unilateral hip OA.

Methods: This study enrolled 57 female patients with unilateral end-stage hip OA. Using computed tomography (CT) taken for arthroplasty planning, muscle composition of the glutei medius and minimus (Gmed+min) and the gluteus maximus (Gmax) was evaluated on axial slice at the lower end of the sacroiliac joint. CT software calculated the cross-sectional area of the segmented total muscle group (TM). Cross-sectional areas of lean muscle mass (LMM), low-density lean tissue (LDL), and intramuscular fat (mFAT) within each TM were calculated using the software based on CT densities. Intramuscular adipose tissue (IMAT) was defined as the summation of the areas of LDL and mFAT. Correlation coefficients were calculated to determine associations between muscle composition variables and ages.

Results: In Gmed+min, TM, LMM, and LMM/TM decreased on the affected side compared with the contralateral side. LDL, mFAT, IMAT, LDL/TM, mFAT/TM, and IMAT/TM increased on the affected side compared with the contralateral side. In Gmax, TM, LMM, and LMM/TM reduced on the affected side compared with the contralateral side. mFAT, IMAT, LDL/TM, mFAT/TM, and IMAT/TM elevated on the affected side compared with the contralateral side. There was no difference in Gmax LDL between the two sides. In Gmed+min on the affected side, LDL, mFAT, IMAT, LDL/TM, and IMAT/TM correlated positively with age. In Gmed+min on the contralateral side, LDL, mFAT, IMAT, LDL/TM, mFAT/TM, and IMAT/TM correlated positively with age while LMM/TM associated negatively with age. In Gmax on the affected side, LDL, IMAT, LDL/TM, and IMAT/TM correlated positively with age while LMM/TM associated negatively with age. In Gmax on the contralateral side, LMM and LMM/TM associated negatively with age while LDL, mFAT, IMAT, LDL/TM, mFAT/TM, and IMAT/TM correlated positively with age.

Conclusion: This study revealed decreases in TM and LMM and increases in fatty infiltration in the hip abductor muscles on the OA-affected side. Increased fatty infiltration in the hip abductors was associated with age on both sides.

Po5_1.45

Total hip arthroplasty induced bullous pemphigoid with subsequent prosthetic joint infection: a case report and review of the literature

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Abstract

Background. Bullous pemphigoid (BP) is an autoimmune disease of the skin characterized by blisters developing in the subepidermal location and caused by autoantibodies that act on the basement membrane. However, BP has been noted in association with several other medical issues and interventions but it is rare following THA. In fact, there are very few accounts of BP in association with a subsequent PJI, which further complicates the diagnosis and treatment.

Case Presentation. Our case is an 88-year-old male, with controlled arterial hypertension, chronic renal insufficiency and early Alzheimer's Disease who underwent an uncomplicated total hip arthroplasty with direct anterior approach for a femoral neck fracture. Tense bullae, which were pruritic, developed at the level of the surgical site at 5 weeks postoperatively, suggesting BP, which was then confirmed by histopathology and direct immunofluorescence. However, 5 weeks after the initiation of treatment, the patient had been experiencing symptoms suggestive of prosthetic joint infection for 2 weeks, including fever, erythema, and joint effusion. Joint aspiration revealed *Staphylococcus Aureus*, confirming PJI.

Discussion. This paper presents a rare but important case of BP and PJI following THA. BP can develop after surgical stress or because of alterations in the immune system induced by the implant and may therefore worsen the skin's integrity and increase the risk of infection. Given the time at presentation, the patient underwent surgical debridement, and the appropriate selection of antibiotics. Subsequently, the patient underwent implant removal and spacer positioning 3 weeks after debridement because of persistent signs of PJI. Three months after the last surgery, antibiotics and no more BP reactions, the patient is scheduled for the second stage reimplantation.

Conclusion. This case highlights the importance of staying alert to unusual skin conditions that arise after joint replacement surgery, as they may signal a deeper problem, like the coexistence of bullous pemphigoid (BP) and prosthetic joint infection (PJI). Successfully managing such cases requires a team approach, ensuring both infection control and the careful treatment of autoimmune complications. Additionally, more research is needed to better understand the connection between BP and joint infections, which could improve care for future patients.

Po5_1.47

Effects of Total Hip Arthroplasty for Secondary Coxarthrosis Due to Developmental Dysplasia of the Hip on the Knee Joint

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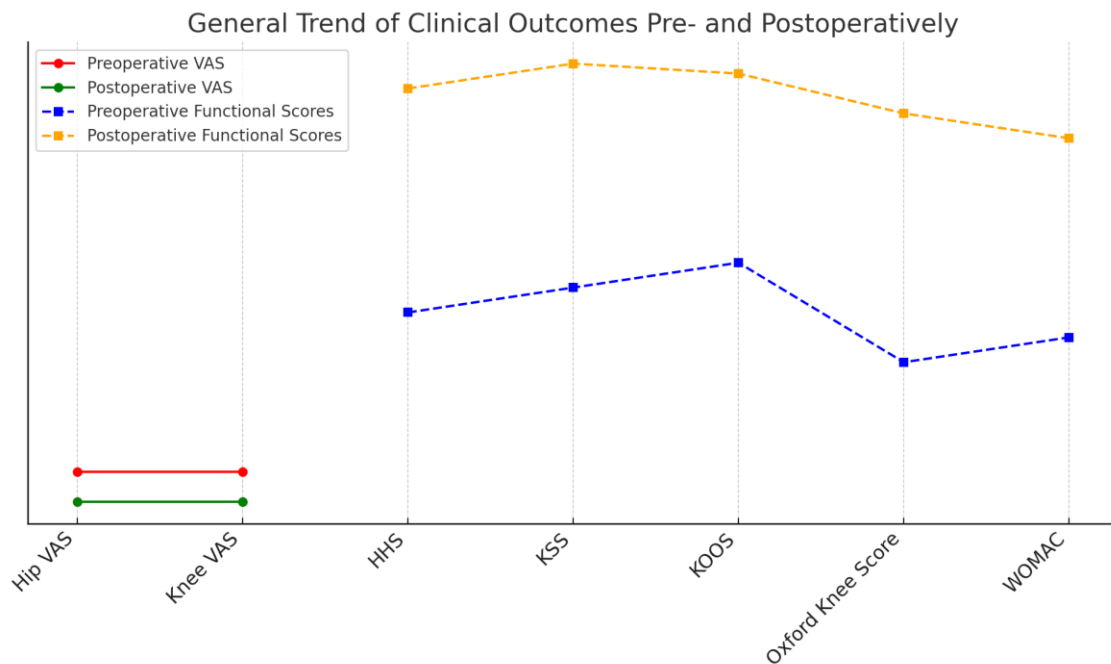
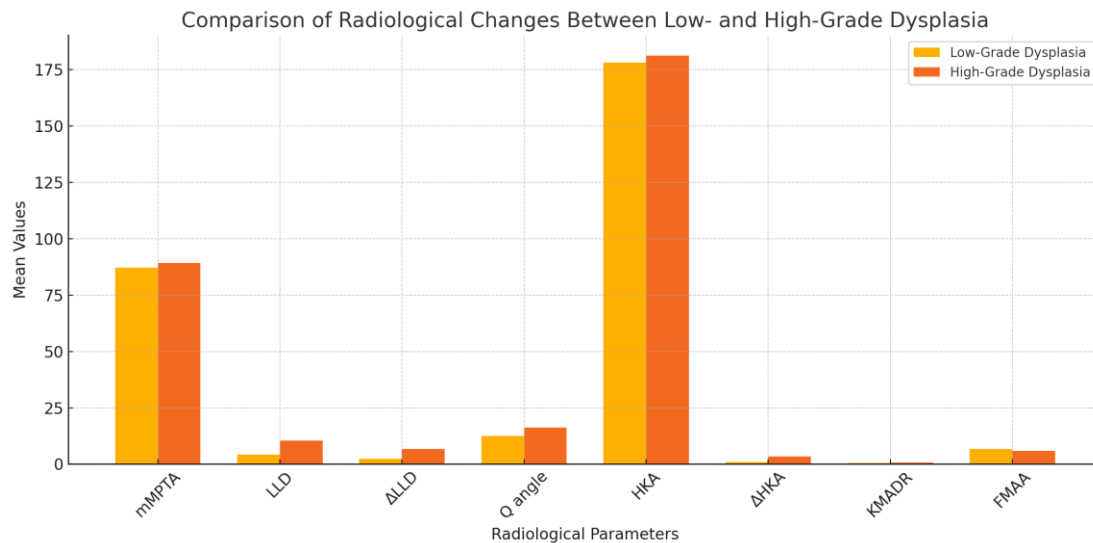
Abstract

Objective: This study aims to evaluate the impact of total hip arthroplasty (THA) performed for secondary coxarthrosis due to developmental dysplasia of the hip (DDH) on lower extremity biomechanics, coronal plane alignment, and knee joint function. Postoperative biomechanical changes in the knee were assessed both radiologically and functionally over a mid-to-long-term follow-up.

Methods: The study included 108 hips, with 74 undergoing unilateral and 34 undergoing bilateral THA. Hips were categorized based on Crowe classification (low-grade: types 1–2; high-grade: types 3–4) and the type of femoral prosthesis used. The mean follow-up period was approximately 4 years (range: 1–10 years). Radiological assessments included mechanical and anatomical lateral distal femoral angle (mLDFA, aLDFA), mechanical medial proximal tibial angle (mMPTA), joint line convergence angle (JLCA), pelvic tilt (PT), leg length discrepancy (LLD), Q angle, femoral offset (FO), hip-knee-ankle angle (HKA), knee mechanical axis deviation ratio (KMADR), femoral mechanical-anatomical axis angle (FMAA), Kellgren-Lawrence (KL) grading for knee osteoarthritis, and tibiotalar angle (TTA). Functional outcomes were assessed using the Visual Analog Scale (VAS), Harris Hip Score (HHS), Knee Society Score (KSS), Oxford Knee Score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC).

Results: The study evaluated 57 hips with low-grade and 51 with high-grade dysplasia. Postoperatively, the high-grade dysplasia group exhibited significantly greater mMPTA, LLD and its change, Q angle, HKA angle and its change, KMADR, and knee KL grade progression, while preoperative and postoperative FMAA values were significantly lower. Among prosthesis types, the lowest mLDFA and FMAA and the highest HKA angle and PT were observed in conical stems, whereas the highest mLDFA, FO, and FMAA and the lowest PT and HKA angle were found in wedge stems. However, no significant differences in clinical outcomes were observed based on Crowe classification or femoral stem type. Postoperatively, all patients exhibited a significant reduction in hip and knee VAS, along with improvements in HHS, KSS, KOOS, Oxford Knee Score, and WOMAC scores.

Conclusion: Following THA, valgus alignment of the knee increases in high-grade dysplasia patients, whereas in low-grade dysplasia cases, this tendency normalizes over time. Conical femoral stems may contribute to increased valgus alignment, while wedge stems provide more stable mechanical axis alignment. Nevertheless, postoperative radiological changes in the knee joint do not significantly impact mid-to-long-term functional outcomes.



Po5_1.48

Bone biomarkers and radiographic segmental weight bearing OA diagnostic scores as biomarkers for canine OA management

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Abstract

Cranial cruciate ligament (CrCL) disease is a highly prevalent orthopaedic disease in dogs causing pain, lameness and osteoarthritis (OA). α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA) glutamate receptor (GluR) antagonists alleviate arthritic degeneration and pain in rodents influencing bone turnover and interleukin-6 (IL-6) (Flood et al., 2007, Bonnet et al., 2015, Bonnet et al., 2020). Correlations between radiographic OA score, glutamate, IL-6 and bone remodelling biomarkers were investigated in dogs with CrCL disease.

Synovial fluid (SF) and serum biomarker concentrations of IL-6, glutamate, receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin (OPG) (Cohort 1), or cartilage oligomeric matrix protein (COMP), procollagen type I N-terminal propeptide (PINP) and cross linked C-telopeptide of type I collagen (CTXI) (Cohort 2), pre-operative weight, total radiographic OA score across 15 measure points (OA score range 15-60) and weight bearing segments of OA score reflecting mediolateral caudodistal femur (MLCaDiFem), mediolateral caudoproximal tibia (MLCaPrTib) and caudocranial proximal tibia (CaCrPrTib) were analysed.

Simple linear regression showed that weight inversely varied with concentrations of RANKL (SF $P=0.0045$; serum $P=0.0437$) and OPG (SF $P=0.0165$; serum $P=0.0208$) (Figure 1 A-D). OA scores positively correlated with SF concentrations of PINP ($P=0.0082$) and CTXI ($P=0.0009$) (Figure 1 E-F).

Spearman analysis for Cohort 1 revealed that the more cranial OA scores (points 9 and 10) were negatively correlated with SF and serum RANKL and OPG concentrations (r_s -0.14 to -0.62, Figure 2A, yellow rectangle). The lateral OA score (point 12) positively correlated with SF RANKL ($r_s=0.84$, $P=0.03810$) and serum OPG ($r_s=0.84$, $P=0.03810$), and inversely correlated with serum glutamate concentration ($r_s=-0.87$, $P=0.02857$). Weight negatively correlated with SF RANKL ($P=0.00476$) and serum RANKL concentrations ($P=0.06627$), and with SF OPG ($P=0.06627$) and serum OPG ($P=0.03413$) concentrations, with r_s ranging between -0.75 to -0.94 (Figure 2A).

Spearman analysis for Cohort 2 revealed that cranial OA score (point 9) negatively correlated with concentrations of PINP (SF $r_s=-0.82$, $P=0.02381$; serum $r_s=-0.82$, $P=0.02381$) and

serum CTXI (rs=-0.82, P=0.02381, Figure 2B, yellow rectangle), lateral OA score (point 12) positively correlated with SF concentrations of PINP (rs=0.71, P=0.04233) and CTXI (rs=0.84, P=0.00794), and OA point 8 positively correlated with SF PINP (rs=0.77, P=0.03175) and CTXI (rs=0.84, P=0.00794) (Figure 2B).

In conclusion, relationships between bone biomarkers and radiographic segmental weight bearing OA diagnostic readouts reveal potential diagnostic or treatment efficacy biomarkers for canine OA management.

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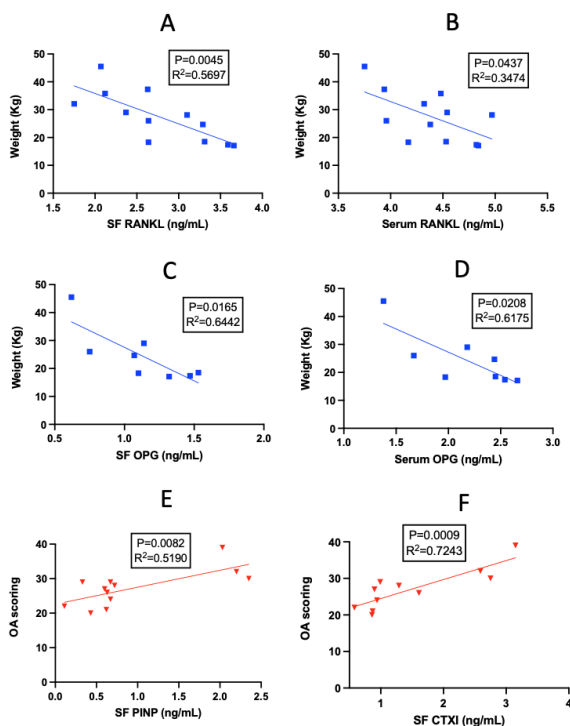


Figure 1 – Significant variations between RANKL (A and B) and OPG (C and D) concentrations in SF and serum with weight, respectively (Cohort 1). Significant variations between PINP (E) and CTXI (F) concentrations in SF with OA score (Cohort 2). Simple linear regression (P<0.05).

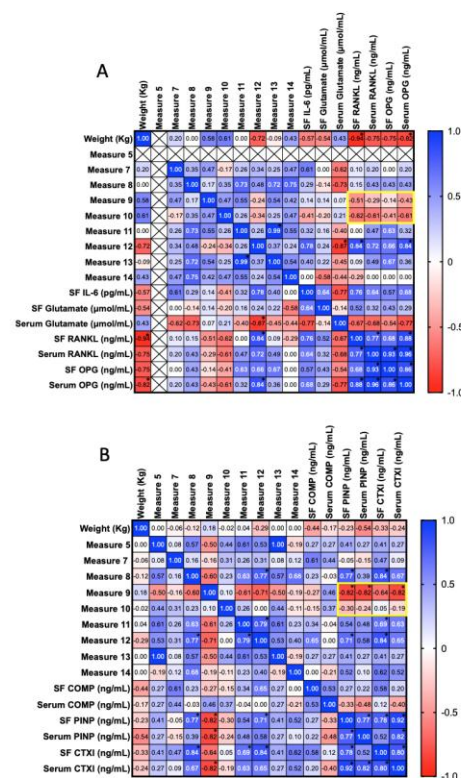


Figure 2 – Correlation matrix analysis in Cohort 1 (A) for IL-6, glutamate, RANKL and OPG concentrations (n=7) and in Cohort 2 (B) for COMP, PINP and CTXI concentrations (n=9), in SF and serum, with OA scores subdivided in three weight bearing segments (MLCaDiFem, MLCaPrTib and CaCrPrTib). The correlation coefficient (rs, nonparametric Spearman correlation) ranges from -1 (red) to +1 (blue). Significant correlations (P<0.05) are marked with asterisks.

Po5_2.01

Translational osteochondral repair in a minipig model

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Abstract

Introduction: The repair of deep osteochondral joint surface defects represents a significant unmet clinical need as untreated lesions often lead to osteoarthritis. The current strategies to repair these defects include osteochondral transplantation or “sandwich” strategies combining bone autografts with autologous chondrocyte implantation, with poorly documented long-term outcomes. In this preclinical study, we investigated the use of a bilayered construct to repair osteochondral defects in skeletally mature minipigs.

Material and methods: Aachen Minipigs were included in this study, creating two defects in the trochlear groove of the right knee. The animals were assigned to four treatment groups namely, a negative control (empty defect), a positive control (autologous osteochondral graft) and two treatment groups (the same bilayered construct but implanted for 16 or 52 weeks).

During the first surgery, defects were created and cartilage sampled for cell culture. During 6 weeks, autologous articular chondrocytes (pAC) were used to create micromasses for the cartilage layer. For the bone layer, we used bioceramics coated with growth factor. During the second surgery, defects were debrided, removing the fibrous ingrowth and cartilage flow, and the bilayered construct was implanted.

After sacrifice, a macroscopic scoring, a contrast-enhanced nanoCT assessment, as well as a histological scoring were performed on all samples.

Results: The negative control, filled with fibrous tissue, showed some cartilage flow at the edges but lacked organized cartilage. The positive control revealed preservation of cartilage and bone but lateral integration of the cartilage layer in the surrounding native cartilage was absent. No upcoming bone front or loss of part of the cartilage layer was observed. The bilayered constructs demonstrated excellent lateral integration of the micromasses of pAC into the native cartilage, with no displacement of the bone front. New bone formation was evident, with almost complete resorption of the bioceramics. In the 52-week group, the formation of a new tidemark between the cartilage and bone was observed, further indicating successful repair.

Conclusion: Bilayered constructs consisting of a cell-based cartilage layer and bioceramics for the bone part display/mimic an organized structure comprising of different tissue forming units to treat deep osteochondral defects of the knee. These constructs could partially recapitulate the biology as seen in the autologous osteochondral graft with the added benefit of good lateral integration of the bilayered construct and even formation of a tidemark after 52 weeks. This strategy shows potential for treating osteochondral defects with enhanced healing, offering benefits over current treatments.

Po5_2.02

Influence of different distraction regimes on femoral bone regeneration in a rat model

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Abstract

Introduction: Huge bone defects are a major problem in orthopedic and trauma surgery. The “gold standard” for human patients with such defects is the callus distraction with a rate of 1 mm/day. Especially for huge defects, this can be very time consuming and increases the risks for complications, such as infections. Therefore, it is necessary to accelerate callus mineralization and further investigate effects of different distraction rates on bone regeneration. The aim of the project was the comparison of two new distraction regimes with the standard.

Materials and Methods: 12 weeks old, male Brown Norway rats received an osteotomy of the diaphyseal femoral bone fixed by an external distraction fixator. Seven days after operation, the animals were subdivided into three regimes with distraction over 12 days and a total elongation of 9 mm. Group 1 represented the clinical standard with constant distraction of 0.75mm/day and therefore served as control. In group 2, the first six days distraction was “fast” (1 mm/day) and another six days “slow” (0.5 mm/day). In group 3, the “fast” and “slow” distraction rates were inverted. Sacrifice was performed 14, 20, 33, and 47 days after operation. The distracted regenerates were analyzed with μ CT to determine BV/TV, trabecular thickness, number, and spacing, as well as density and relative volume of hard, soft, and complete callus. Next, the bones will be analyzed histologically for callus mineralization, cartilage, vascularization, osteoblasts, -clasts, IL-6 and β -Catenin. Molecular analyses of the regenerate will be performed via Micro Array, validated by qRT-PCR and Western Blot, to investigate potential alterations in signaling pathways as for BMP2/4, HIF1a, IL-6, OC, RUNX2, RANKL/OPG, SMAD, TGF β , VEGF and Wnt genes. Bone metabolism markers (OPG, RANKL, CTX, Tracp5b, alkaline phosphatase and osteocalcin) will be measured systemically within the plasma via ELISA.

Results: All animal experiments were completed successfully without differences between the distraction regimes in physiological parameters such as activity, lameness or weight progression. The bone morphometric parameters revealed no significant differences between the three regimes at any time point. The relative volume of the complete callus was significantly increased in group 3 (27,93 $\pm$ 6,37%) on day 33 compared to group 1 (18,93 $\pm$ 6,37%; p=0.0149). Further analyses are currently ongoing.

Conclusion: Radiologically, the new regimens showed results as good as the standard regimen of constant distraction, meaning that further analyzes at the histological and molecular levels could provide promising findings for clinical use.

Po5_2.03

Anti-RANKL treatment attenuates sarcopenia via modulating mitochondria and macrophage infiltration

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Abstract

INTRODUCTION: Sarcopenia is characterized by progressive loss of skeletal muscle mass, strength, and muscle function associated with typical aging. People with sarcopenia are associated with poor balancing abilities and substantial increase in fall risks that may result in increased fragility fracture rate. The increase in chronic low-grade systemic inflammation during aging is associated with sarcopenia. The receptor activator of nuclear factor NF- κ B ligand (RANKL) is expressed in skeletal muscle and its activation mainly inhibits myogenic differentiation, which leads to skeletal muscle dysfunction. Previous study showed that RANKL inhibition could improve muscle strength and restore bone mass in adult osteoporotic mice, indicating a great potential of RANKL inhibition for treating sarcopenia. The study aims to investigate the effects of anti-RANKL treatment on sarcopenic skeletal muscle and explore the related mechanisms in terms of mitochondria modulation and the polarization status of macrophages.

METHODS: Sarcopenic senescence-accelerated mouse P8 (SAMP8) mice at month 6 were treated intraperitoneally with 10mg/kg anti-RANKL (Bio X Cell) every 2 weeks and harvested at month 10. Senescence accelerated mouse resistant-1 (SAMR1) were collected at month 10 as age-matched non-sarcopenic group. Ex-vivo functional assessment, grip strength, oil red O and immunostaining of CD45, F4/80, CD206, iNOS, C/EBP α , and Pax7 were performed. Mitochondria morphology was examined with a transmission electron microscope (Hitachi H7700, Tokyo, Japan). One-way ANOVA analysis was done, and the significant level was set at $p \leq 0.05$.

RESULTS: After anti-RANKL treatment, tetanic, twitch force and grip strength were significantly higher than CTL group ($p < 0.01$, $p < 0.01$ and $p < 0.05$). The SAMP8 mice at month 10 expressed significantly more C/EBP α (intramuscular adipose marker), CD206 (M2 marker) and LYVE1 (macrophage marker) positive area than in SAMR1. Anti-RANKL treatment could significantly decrease CD45 (general leukocyte marker), F4/80 (M1 marker), iNOS (M1 Marker), C/EBP α and CD206 positive area and oil red O area, and significantly increase PAX7 (MDSC marker) positive cell numbers. There was an increase in the number of intermyofibrillar mitochondria and restoration of mitochondria morphology in anti-RANKL group. The structure of mitochondria and their cristae were more compact, compared with swollen mitochondria in the isotype group.

DISCUSSION: These results indicated an excessive inflammation and the promoted shift from M1 to M2 in macrophage phenotype during sarcopenia. The anti-RANKL treatment protected against sarcopenic skeletal muscle through suppressing muscle inflammation and modulating mitochondria which may represent a novel therapeutic approach for sarcopenia.

Po5_2.04

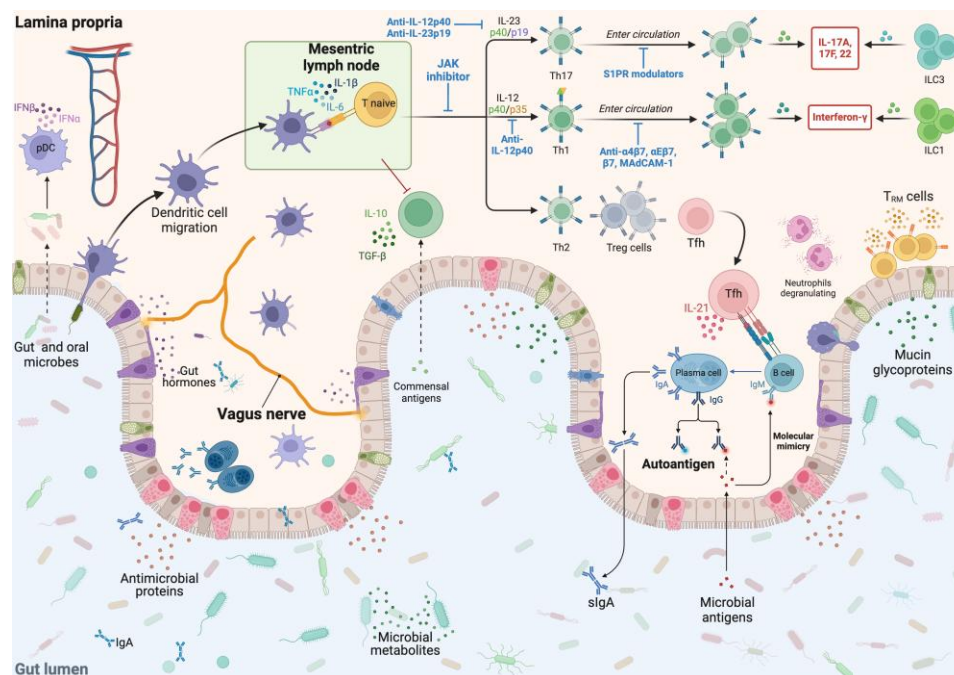
The Role of the Gut-Bone-Brain Axis in Degenerative Shoulder Diseases: Mechanisms and Implication

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Abstract

Degenerative shoulder diseases, including rotator cuff injuries and osteoarthritis, are leading causes of pain, disability, and diminished quality of life, representing a significant burden on individuals and healthcare systems globally. Recent research has begun to explore novel mechanisms that underlie these conditions, and one promising area of investigation is the gut-bone-brain axis. Emerging evidence suggests that interactions between the gut microbiota, bone metabolism, and neurological pathways may play a crucial role in the progression of shoulder degenerative diseases. Specifically, gut microbiota composition influences systemic inflammation, immune responses, and metabolic processes that affect both bone health and neural function. Additionally, changes in bone metabolism and neural signaling can impact gut health, creating a bidirectional regulatory loop. In this review, we provide an in-depth analysis of the role and mechanisms of the gut-bone-brain axis in shoulder degenerative diseases. By highlighting the intricate connections between these systems, we aim to offer a comprehensive understanding of how gut and brain health may influence the progression of degenerative shoulder diseases, ultimately providing new insights for preventive and therapeutic strategies in this field.



Po5_2.05

**The effect of rotational guided growth with flexible tethers on the distal femoral bone.
An experimental study in a porcine model.**

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Abstract

New and innovative surgical techniques, using a flexible tether to correct rotational deformities of the growing femur have recently emerged, but are lacking radiographic validation of desired rotation and adverse effects in the frontal and sagittal planes. We therefore investigated this new technique in an established large animal model.

Eight skeletally immature pigs were included in the study in a paired (left vs. right leg) design. Mean (range) observation time was 83 days (77-90). CT-scans and X-rays were performed immediately after insertion of the tethers and again at euthanasia after twelve weeks. From the CT-scans, 3D-models of the left and right femora were generated and analyzed for intended external rotation and unintended effects in other planes and bone morphology. One pig was excluded due to surgical site infection.



Fig.1: On top is the Hinge Plate used before separation.

Bellow, two Hinge Plates have been separated and the male parts connected by double looped Fibertape.



Average change in rotation between the operated and non-operated femora was 7.2° (CI: 5.9 - 8.6) external rotation. We also found unintended valgus deformity in the coronal plane (7.8° (CI: 3.9 – 12.0) and unintended extension in the sagittal plane (3.9° (CI: -1.8 – 8.7). Furthermore, unintended changes to the joint morphology and abundant tissue overgrowth covering the tethers, were also observed. Rotation by guided growth was achieved with this surgical technique, confirmed by radiological measurements.

Clinical significance. While this large animal study has shown that this new surgical technique reliably corrected rotational deformities by guided growth, it has also revealed adverse effects that need further investigation before clinical use.

Fig. 2: Picture showing groove in the bone after tissue overgrowth. Note that bone and tissue have been removed prior to taking the photo to free the tether, which has been removed.

Po5_2.06

Influence of fixation methods on macrophage expression and inflammatory cytokines in induced membranes during the Masquelet technique: A comparison of plate and interlocking intramedullary nail fixation in a mouse model

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Abstract

Introduction. In the Masquelet technique, induced membranes formed around polymethylmethacrylate (PMMA) spacers play a crucial role in bone regeneration. Macrophages within these membranes have been suggested to significantly contribute to the bone healing process. However, studies investigating the impact of fixation methods for bone defects on the properties of induced membranes remain limited. This study aimed to examine the effects of fixation methods on the expression of inflammatory cytokines and macrophages in induced membranes.

Method. Thirty 6-month-old male C57BL/6J mice were divided into two groups: the plate fixation group (P group, n=15) and the intramedullary nail fixation group (IM group, n=15). A 2-mm critical-sized bone defect was created in the mid-femur, and a PMMA spacer was inserted into the defect in both groups. In the P group, the femur was stabilized using a locking plate and screws, while in the IM group, stabilization was achieved using an intramedullary nail and locking pins (Fig 1). Tissue samples were collected on postoperative days 3, 7, and 14. The expression of inflammatory cytokines (IL-1 β and IL-10) and macrophage markers (iNOS for M1 macrophages, CD163 for M2 macrophages) was evaluated using quantitative polymerase chain reaction (qPCR).

Result. Induced membranes formed around the spacers in both groups, but the IM group exhibited macroscopically thicker membranes (Fig.1). The expression levels of IL-1 β and IL-10 were highest on day 3 and decreased over time. The P group showed significantly higher expression levels of both cytokines compared to the IM group on days 3 and 7. Regarding macrophage markers, iNOS expression was significantly higher in the P group on day 3, while CD163 expression was significantly higher on days 7 and 14 (Fig 2).

Discussion. This study demonstrated that the fixation method for bone defects influences the expression of inflammatory cytokines and macrophages in induced membranes. Plate fixation was associated with higher expression of inflammatory and regenerative macrophages compared to interlocking nail fixation. Although this study does not evaluate the ultimate outcome of Masquelet's second-stage bone grafting and bone healing, the

induced membranes in the P group may possess more favorable characteristics for bone regeneration.

Conclusion. Macrophage expression in induced membranes during the Masquelet technique is influenced by the fixation method for bone defects. Plate fixation may generate induced membranes that are more advantageous for bone regeneration than those produced by interlocking nail fixation.

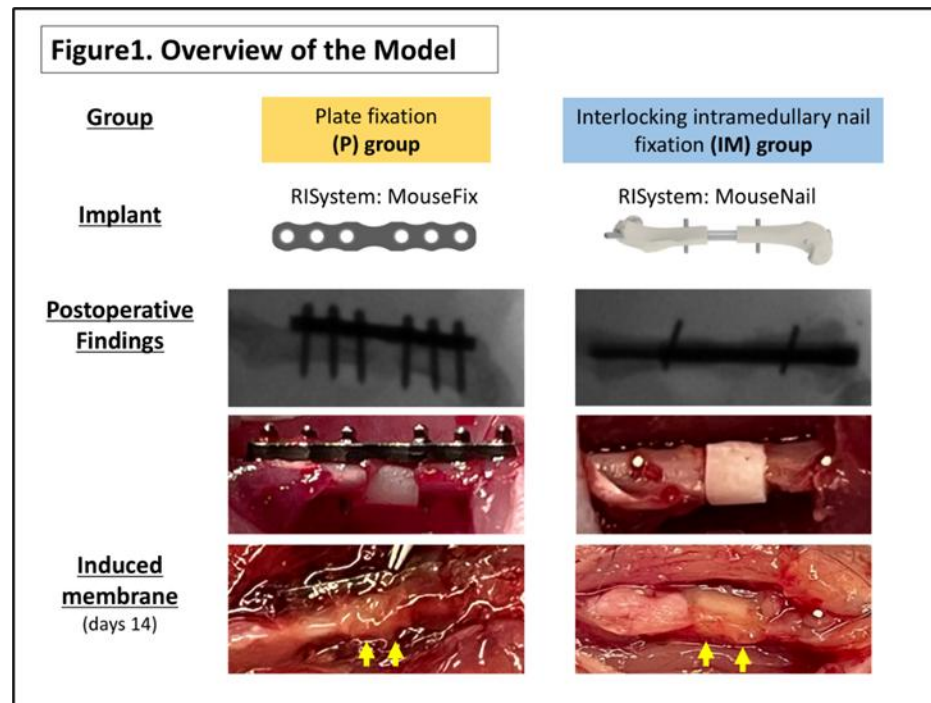
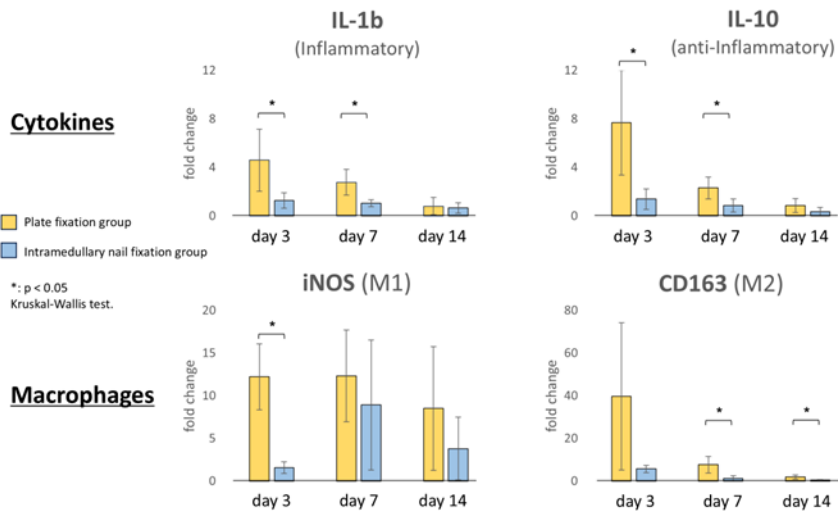


Figure2. mRNA Expression (qPCR)



The expression of cytokines and macrophages within the induced membrane tended to be higher in the plate fixation group

Po5_2.07

Investigating magnesium's role on murine bone in obese condition

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Abstract

Magnesium (Mg) plays a pivotal role in bone metabolism and its deficiency leads to decreased bone mass and brittle bones. Various studies demonstrated an elevated risk of bone fractures in obese children and adolescents. Actually, the impact of hypomagnesaemia on fracture risk remains uncertain. Here we aimed to investigate the effects of Mg-deficiency on the systemic inflammatory processes and bone morphology under diet-induced obesity in a juvenile, growing mouse model. Therefore, 8 weeks old male C57BL/6J (WT) mice were randomly assigned to four different dietary groups including high-fat with/without Mg- reduction and low-fat with/without Mg-reduction. Diet-induced obesity was assessed by frequent body weight and fasting glucose measurements, glucose- and insulin tolerance tests as well as monitoring of lipid parameters. After 18 weeks, animals were sacrificed. Bone morphology was structurally investigated using μ CT, EDX and SAXS. We demonstrated improved inflammatory and metabolic parameters of Mg²⁺-deficient animals with diet-induced obesity compared to their high-fat controls. Additionally, in the bone the Mg-deficiency indicated diminished immunological activities within the marrow and structural changes of the hydroxyapatite crystals. Investigation of immune cells (e.g. macrophages) in the bone tissue are currently under investigation. However, the Mg content of the bone exhibited no alterations in the context of diet-induced obesity. In conclusion, the detrimental outcome of diet-induced obesity was limited when fed a Mg-reduced high-fat diet in juvenile, growing mice indicating a protective role of Mg-deficiency under obese conditions.

Po5_2.09

Triamcinolone Hexacetonide: Time- and Dose-Dependent Balancing Between MMPs and TIMPs in OA-Driven Synovial Inflammation

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Abstract

Introduction: Inflammation drives osteoarthritis (OA) progression, with cytokines like IL-1 β , IL-6, and TNF- α orchestrating the inflammatory cascade in synovial cells. These cytokines amplify the production of matrix metalloproteinases (MMPs), potent enzymes responsible for extracellular matrix breakdown in cartilage. In contrast, tissue inhibitors of metalloproteinases (TIMPs) serve as critical regulators, counteracting MMP-activity to maintain matrix integrity. While intra-articular corticosteroids, such as triamcinolone hexacetonide (THA), are a mainstay for their anti-inflammatory effects and prolonged action with minimal systemic impact, their influence on the intricate MMP-TIMP balance remains unresolved. This in-vitro study investigated the time- and dose-dependent effects of THA on cytokine release, MMP activity, and TIMP production in synovial cells from healthy and OA-affected patients.

Methods: Human synovial cells isolated from OA and healthy donors (N=6) were treated with three concentrations of triamcinolone hexacetonide (THA; 0.1 μ M, 0.01 μ M, and 0.001 μ M) for 1 and 7 days, alongside untreated controls. Cell viability was assessed qualitatively using live-dead assays and quantitatively via MTS assays to determine the optimal THA dosage. Structural changes in the cytoskeleton were visualized through F-actin cytoskeleton labeling. Gene expression profiles were analyzed using qPCR to evaluate changes in cytokines (IL-1 α , IL-1 β , IL-6, and TNF- α), MMPs (MMP-1, -3, and -13), and TIMPs (TIMP1-4) between OA and healthy synovial cells, with and without exposure to THA (0.01 μ M).

Results: THA induced a dose-dependent reorganization of F-actin and a notable reduction in cell count, particularly at 0.1 μ M and 0.01 μ M concentrations. Consistently, the MTS assay revealed a highly significant decrease in cell viability between the control and both 0.1 μ M and 0.01 μ M THA in healthy and OA synovial cells ($p < 0.0001$), while the lowest concentration (0.001 μ M) exhibited a less pronounced effect ($p = 0.03$). Gene expression analysis demonstrated a significant impact of THA, showing suppression of pro-inflammatory cytokines and MMPs, alongside an upregulation of TIMPs (1, 2, and 4) at 0.01 μ M on days 1 and/or 7 within both groups. Cross-group comparisons highlighted significantly increased expression of MMP1 (Day 7, $p < 0.0001$), MMP3 (Day 7, $p < 0.01$), and TIMP3 (Day 1, $p < 0.001$), with a concurrent reduction in TNF- α expression (Day 1, $p < 0.05$) in OA synovial cells.

Conclusion: THA induces dose-dependent cytoskeletal reorganization and reduces cell viability, particularly at higher concentrations. It suppresses pro-inflammatory cytokines and MMPs while upregulating TIMPs in a dose-dependent manner, with distinct effects in OA synovial cells, highlighting its potential to modulate inflammation and matrix degradation and regulation in OA.

Po5_2.10

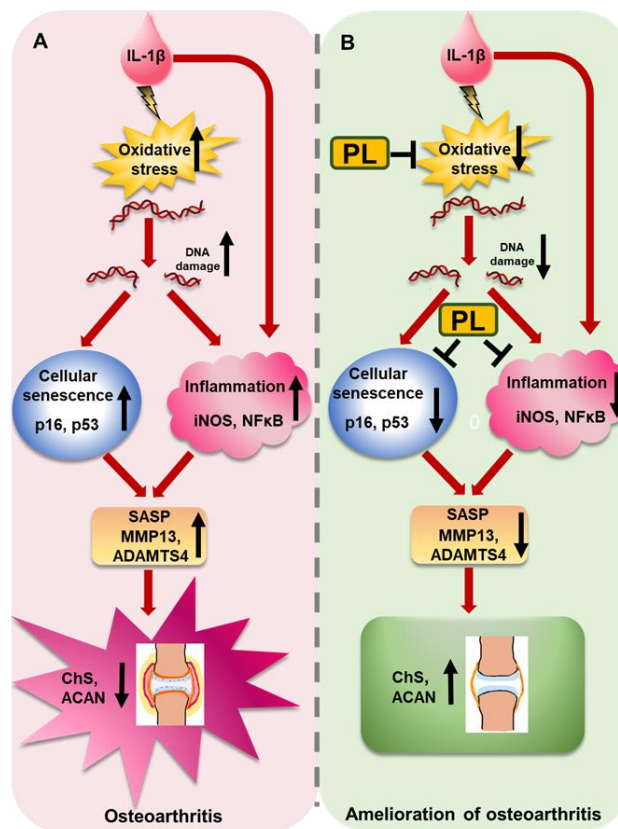
Piperlongumine mediates amelioration of OA via inhibition of chondrocyte senescence and inflammation

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Abstract

Osteoarthritis (OA) is a degenerative joint disease that adversely affects the daily lives of affected individuals. With aging, chondrocyte senescence sets in, resulting in a vicious signaling loop that aids the progression of the disease. Efforts in the quest for pharmaceuticals have been in vain, as most of the potential disease-modifying OA drugs (DMOADs) have failed to progress beyond clinical trials. This void motivated us to identify new DMOADs that target senescence for the treatment of OA. In this study, we first confirmed the suitability of the goat ex vivo OA model (emphasizing 3R's principle) for the screening of senotherapeutics, namely, ABT-263, ABT-737, and Piperlongumine (PL), wherein PL showed a positive outcome in the preliminary studies. Thereafter, we determined the cytocompatible concentrations of PL using live/dead staining of chondrocytes. Treatment of goat ex vivo OA cartilage with PL exhibited a concentration-dependent increase in the retention of key cartilage matrix components. We then examined the effect of PL on chondrocyte senescence and observed a decreased expression of major senescence markers in the PL treated groups. Interestingly, PL treatment reduced the expression of major downstream effectors of the chondrocyte senescence pathway in a concentration dependent manner. Moreover, IL1 β -induced elevated levels of oxidative stress and DNA damage in cartilage explants were rescued by all the tested concentrations of PL. In addition, PL also exhibited an anti-inflammatory effect and decreased the expression of NF κ B and iNOS in the goat OA cartilage explants. Finally, we proposed a model for the mechanism of action of PL in the treatment of OA. Overall, PL showed a promising outcome as a senotherapeutic for the amelioration of OA in the goat ex vivo OA model.



Keywords: Chondrocyte senescence, Senotherapeutics, Osteoarthritis, Piperlongumine, Disease modification. *Schematic illustration of the proposed mechanism of action of Piperlongumine in OA amelioration.*

Po5_2.11

Reducing pin track infections by using implants with different coatings and antibiotic release

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Abstract

Background: A myriad of factors are accountable for the prevalence of pin track infections in external fixation. An impeding part of infection prevention is the lack of standardized pin site care management. The methods of prevention vary greatly, with use of different pin materials, coatings, and antimicrobial solutions. The aim of this preclinical study was to determine the possibility of preventing pin track infections by using implants with different structures and antibiotic coatings.

Materials and Methods: The pins used in the study were either anodized, nitrided or porous, with and without a gentamicin antibiotic coating. The contamination was performed with a strain of *Escherichia coli*, being sensitive to gentamicin.

Six types of pins were tested in a total of 72 Wistar rats implanted with pins of different structures and antibiotic coatings. The animals are assigned to 7 groups as follows.

Group 1 (control): 12 animals divided in three subgroups and implanted with either anodized (4 rats), nitrided (4 rats) or porous (4 rats) pins without antibiotic coating and without bacterial contamination.

Group 2: 12 animals implanted with anodized pins without antibiotic coating but with bacterial contamination.

Group 3: 12 animals with antibiotic-coated anodized pins and bacterial contamination.

Group 4: 12 animals with nitrided pins without antibiotic coating but with bacterial contamination.

Group 5: 12 animals with antibiotic-coated nitrided pins and bacterial contamination.

Group 6: 12 animals with porous pins without antibiotic coating but with bacterial contamination.

Group 7: animals with antibiotic-coated porous pins and bacterial contamination.

All pins were removed and examined 14 days after implantation.

Results: No bacterial growth was observed in Group 1, 3 and 5, while the bacterial growth in the nitrided pins of Group 4 was 10%. In the remaining groups the bacterial growth was over 20%.

Conclusions: The results in terms of bacterial growth were comparable between the antibiotic-coated pins and those with a nitrided structure. The use of pins with a nitrided structure and those with an antibiotic coating can significantly reduce the risk of developing a pin track infection.

Po5_2.13

ACLT + pMMx surgery successfully induces post-traumatic osteoarthritis in sheep

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Abstract

Background: Reliable animal models are crucial to demonstrate the efficacy of potential disease-modifying osteoarthritis (OA) drugs. A reproducible, low-invasive, fast-developing and moderately severe animal model of OA is the anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy (pMMx) model. Although it has been extensively studied in rodents, its application to sheep remains to be explored. **Objectives:** The goal of the present study was to validate the ACLT-pMMx model as a reliable approach to induce OA in sheep.

Methods: Thirty-two Ile-De-France female sheep were subjected to surgical ACLT + pMMx of the left knee. Twenty-four of them received intra-articular injection of 10 vs. 100 µg/knee HAPLN1 human recombinant protein (rhHAPLN1) or vehicle at week 11 and 19. Macroscopical and histological evaluation of the cartilage, synovial membrane and osteophytes was performed according to the OARSI recommendations on samples collected 27 weeks after surgery. Biomarkers were assessed on synovial fluid (SF) collected at D0, as well as after 11, 15, 19, 23, and 27 weeks.

Results: The synovial membrane showed significant signs of synovitis in the operated knee when compared to the contralateral knee. This was confirmed by the significantly higher SF score 11 weeks post-surgery as well as by the increased total protein content in the SF of the operated knees compared to their contralateral counterparts up to 19 weeks post-surgery. Moreover, macroscopic analyses evidenced clear damage of the cartilage and an increase of osteophytes, with the medial tibial condyle being the most impacted followed by the medial femoral condyle. Interestingly, injection of rhHAPLN1 significantly reduced the macroscopic scores assessing cartilage, osteophytes, and synovial membrane.

Microscopic cartilage alterations in the operated knee were mainly visible on the site of meniscus removal, specifically in the cranial and caudal regions. Increased erosion and fibrillation of the cartilage layer, lower chondrocyte density, and abnormal chondrocyte cell cloning were also observed at the medial tibial condyle. Exacerbated cartilage degradation was confirmed by elevated SF-levels of Coll2-1 up to week 19.

Conclusion: The ACLT + pMMx model effectively induced a moderate to severe OA pathology in sheep after 27 weeks. The medial tibial condyle was the most impacted sub-region due to the removal of the cranial part of the medial meniscus. Synovitis and cartilage degradation were further confirmed by increased total protein content and Coll2-1 levels in the SF of operated knees.

Po5_2.15

A nociceptor predictive model of patient reported pain

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Abstract

Introduction. Osteoarthritis (OA) is one of the greatest contributors to chronic pain worldwide¹. In OA, joint nociceptors develop spontaneous activity and increased excitability and responsiveness to stimulation². The molecular mechanisms governing mechanically induced pathology and pain in OA remain elusive and currently no in vitro models can capture OA changes in nociception.

Hypothesis: Differentiation of human iPSC to joint nociceptors that when stimulated with OA patient synovial fluid samples will reveal changes in activity that correlate with patient reported pain.

Methods. Synovial fluid from 12 subjects with knee OA (KL grade II-IV) undergoing high tibial osteotomy surgery (HTO) or total knee replacement (TKR) was analysed by ELISA for OA pain associated markers and used for nociceptor stimulation. Patient pain and function were evaluated using Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire. Human ioSensory Neurons iPSC (Bit.bio) were plated at 60,000 cells/cm² on Poly-D lysine/laminin coated 24-well micro electrode array (MEA) plates (Axion) (n=24). Neural cultures were maintained in neural basal media with GlutaMAX, B27 and N-2 with neurotrophic factors and doxycycline supplementation for nociceptor differentiation. Media changes were 80% daily (days 1-6), and DOX supplementation halted (day 7), and 50% media changes performed every 2 days (days 11-30) to reduce mechanical perturbation. Nociceptor culture baseline activity was recorded using an Axion Maestro Pro instrument, cultures removed and stimulated with individual patient synovial fluids in duplicate, replaced into instrument and after 20-minutes rest period, cell activity recorded for 5 minutes every 30 minutes for 12 hours. Changes in electrode recording of >6x standard deviations with an interval of <2.16ms were defined as spikes in activity. Data was analysed for normality and equal variance, Pearson's correlation matrices and by Two-way Anova with multiple comparisons using GraphPad Prism 9.

Results. The addition of OA patient SF to nociceptor cultures significantly increased spontaneous activity from baseline in all cultures 30 minutes post transfer. This immediate spike in activity correlated with synovial fluid NGF concentration (r=0.768, p=0.004). The change in nociceptor activity correlated with all patients KOOS sports and recreation subscale scores (r=0.762, p=0.028).

Conclusions. We have developed a novel human cell model of pain that when stimulated with OA patient joint fluid shows changes in activity that correlate with individual patients' pain experience and NGF concentrations.

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Po6_2.01

Effect of age, sex, height, ethnicity, and femoral bowing on the anatomical fitting of the LCP Distal Femur plate

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Abstract

Lateral locking-plate fixation is commonly used for distal femur fractures, and pre-contoured plates attempt to match the bony anatomy of the target patient population. However, plate fit is highly variable due to inter-subject morphological differences. If plate misfit is not recognized and addressed, this can result in axial malalignment. This study evaluates the effects of age, sex, height, ethnicity, and femoral bowing on the anatomical fit of a distal femur plate. Unilateral 3D models of 80 (40 male, 40 female) Caucasian and 79 (34 male, 45 female) Vietnamese femora were utilized. Both cohorts consisted of young (< 65 years) and old ($\geq$ 65 years) subjects, with 40 young and 40 old Caucasian, and 36 young and 43 old Vietnamese. The plate undersurfaces of 9-, 11- and 13-hole LCP Distal Femur plate 3D models were positioned on the bone models and anatomical fitting was assessed by applying developed clinical criteria. Satisfactory plate conformity was achieved from plate head up to hole 6, with most measurement locations fitting 52–100% of bones for both ethnicities. There was proximal plate misfit from hole 8 onwards of 0–41% fit in that region, and a mean distance of 11.6 mm at the proximal plate tip for Caucasians, with a larger misfit (16.3 mm, $p \leq 0.017$) for Vietnamese. Patient sex, height, ethnicity, and femoral bowing all had significant impacts on fit, while the effect of age was limited. Due to sex and ethnicity related height differences, this suggests that patient height and femoral bowing are the main variables affecting fit. The observed proximal plate misfit for both Caucasians and Vietnamese suggests they might not fit as anatomically close as generally assumed. If the plate is used as a reduction tool, pre-operative templating of the intact contralateral femur can potentially identify plate misfit

Po6_2.02

Garden classification of femoral neck fracture using deep-learning algorithm

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Abstract

The Garden classification, based on X-ray interpretation and established over 50 years ago, remains the standard clinical classification system for femoral neck fractures (FNFs). Yet, this classification has a high interobserver variability of 70%. We sought to develop a deep-learning algorithm capable of accurately predicting FNF types, using only X-ray images, with performance comparable to that of computed tomography (CT). We retrospectively collected data from 1,588 patients who underwent X-ray and 3D-CT scans and were diagnosed with femoral neck fractures at Asan Medical Center. The input X-ray dataset consisted of paired X-ray images of the hip, with anteroposterior (AP) and lateral views. Using 3D-CT as the reference standard, patients were labeled as Garden types I (n = 378, 23.8%), II (n = 68, 4.3%), III (n = 477, 30.0%), and IV (n = 665, 41.9%). Our algorithm consisted of hip-joint detection followed by Garden classification, for which 12 different deep-learning architectures were evaluated. Algorithm performance was externally validated in 100 patients. Our algorithms showed a 91.3% overall accuracy, 90.5% sensitivity, 88.6% precision, and 88.9% specificity, indicating excellent FNF type discernment. Our algorithms could serve as a valuable tool for diagnosing FNF based on X-ray data only, with accuracy comparable to that of CT.

Po6_2.03

Preoperative knee flexion muscle strength predicts postoperative daily activity immediately after total knee arthroplasty

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Abstract

The aim of this study is to investigate the relationship between patient-reported outcome measures (PROMs) and patients' daily movements, as evaluated by a wearable physical monitor, and to identify factors associated with improvements in daily life activities following total knee arthroplasty (TKA). In this study, varus knees underwent TKA was evaluated. Daily life activities were assessed using a WPM one month before TKA and from 4 to 6 weeks postoperatively. PROMs were assessed using the 2011 Knee Society Score before and 6 weeks after TKA. Additionally, preoperative limb muscle strength was evaluated using the Cybex system. Preoperative PROMs did not directly predict daily life movements after TKA. After 6 weeks after TKA, total activity recovered to approximately 74% of preoperative levels in this elderly Japanese population. Adequate preoperative flexion muscle strength significantly influences moderate physical activity levels in the early postoperative period.

Po6_2.04

Evaluating Intraoperative Dynamic Hip-Knee-Ankle Angle Under Controlled Load During Navigated Total Knee Arthroplasty

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Abstract

Objective Traditional knee alignment assessment methods in total knee arthroplasty (TKA) rely on static radiographs, which do not account for dynamic changes in hip-knee-ankle (HKA) angles during movement. This can limit the accuracy of personalized alignment techniques, leading to suboptimal outcomes. The motivation of this study is to improve intraoperative dynamic HKA (dHKA) measurements using a novel method that applies a constant distraction force through the knee's flexion arc, ensuring more precise and stable alignment for personalized surgical planning.

Material & Methods This retrospective review included 1790 tibia-first TKA cases performed by 87 surgeons using a proprietary cloud-based computer-assisted orthopedic system (CAOS). Conventional dHKA was acquired by manually manipulating the leg through flexion (0° to 120°), while the proposed method used an intra-articular device to apply a distraction force. Precut and postcut dHKA values from both methods were compared.

Results dHKA differences between the conventional and proposed methods were calculated for both precut and postcut phases. Precut Newton acquisition showed a more neutral alignment compared to conventional precut kinematics, with flatter differential curves observed across the full flexion arc. Postcut measurements showed minor differences, with the proposed method showing slightly less valgus.

Conclusion The novel intra-articular tensioner improves stability during dHKA acquisition, providing more reliable and neutral alignment data. The findings suggest that proposed acquisitions based on intra-articular device can serve as accurate surrogates for conventional kinematic methods. Incorporating dynamic knee phenotypes into surgical planning could further personalize alignment strategies in TKA, leading to better functional outcomes.

Po6_2.05

Open-Access 3D Bone Shape Databases in Orthopedics: An Unmet Need?

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Abstract

Objective: 3D bone shapes play a critical role in preclinical and clinical orthopedic applications. This study aimed to identify and evaluate 10 most relevant existing online CT databases to see if they meet requirements of biomedical experts. Method: We performed a systematic search to identify relevant online CT databases for lower extremities. Additionally, a workshop with n=40 biomedical experts was held to gather insights on the benefits, challenges, and users for an online 3D bone shape database. This information was used to establish criteria to evaluate the identified databases. Results: We found that currently available online databases inadequately address experts' needs, particularly regarding inclusion of different shape formats, such as 3D meshes and CAD models, and inclusion of mechanical properties of bones. Conclusion: These findings highlight a significant gap between databases' offerings and users' needs, underscoring the need for more comprehensive, accessible resources and advanced tools to support the field's progression

Po6_2.06

Computer-simulated corrective osteotomy for malunion after distal radius fracture normalizes bone density distribution in the subchondral radius.

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Abstract

Malunion of distal radius fractures alters joint alignment, mechanical environments, and subchondral bone density, contributing to osteoarthritis. Computer-simulated corrective osteotomy enables precise alignment restoration, but its effects on subchondral bone density remain unclear. This study evaluated subchondral bone density distribution changes in eight women (mean age: 63.3 ± 7.2 years) who underwent computer-simulated corrective osteotomy for malunited distal radius fractures. Pre- and postoperative CT data were used to create 3D bone surface models. Subchondral bone density within 3 mm of the articular surface was analyzed in four regions: volar and dorsal scaphoid fossa, and volar and dorsal lunate fossa. High-density volume (HDV) was defined as areas above the median bone density, and %HDV was compared to pre- and post-correction. Changes in the centroid coordinates of HDV distribution were also evaluated. Post-correction, %HDV increased significantly on the volar side ($p < 0.05$) and decreased on the dorsal side ($p < 0.05$). When comparing normal sides and the corrected, no significant differences in %HDV were observed in any area. Additionally, the centroid of HDV distribution shifted 0.69 mm toward the volar side in the volar-dorsal direction after correction. Corrective osteotomy normalized the subchondral bone density distribution, suggesting improved stress distribution. This may inhibit osteoarthritic progression, highlighting the biomechanical benefits of alignment correction. These findings indicate that computer-simulated corrective osteotomy normalizes subchondral bone density distribution, potentially mitigating osteoarthritis progression and highlighting its biomechanical benefits in malunited distal radius fractures.

Po6_2.07

Surgical outcomes of arthroscopic osteophyte debridement for elbow osteoarthritis using a navigation system

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Abstract

Background: Elbow osteoarthritis often results in restricted joint range of motion (ROM) and endpoint pain due to osteophyte formation and joint contracture. Arthroscopic osteophyte debridement, although minimally invasive, presents challenges in accurately identifying lesions during surgery. This study aimed to evaluate the clinical utility of a surgical system combining 3D computer simulation and a CT-based navigation system for real-time visualization during arthroscopic debridement. Methods: Three patients (two men, one woman; mean age 53.3 years) with elbow osteoarthritis underwent arthroscopic osteophyte debridement using the proposed navigation system. Preoperative CT images were used to create 3D bone models for surgical planning. During surgery, a tracker system synchronized the arthroscopic and navigation monitors, enabling real-time visualization of the osteophyte removal sites. Surgical accuracy and ROM were evaluated postoperatively, with a mean follow-up of 9.6 months. Results: The overall osteophyte removal rate was 86%, with a total excessive removal rate of 12%. The posterior compartment achieved an 89% osteophyte removal rate with no excessive removal, while the anterior compartment achieved an 82% removal rate with a higher excessive removal rate of 27%. Postoperative ROM improved from -32 ± 10 degrees to -15 ± 13 degrees in extension and from 115 ± 10 degrees to 133 ± 8 degrees in flexion. Conclusion: The CT-based navigation system demonstrated high surgical accuracy and favorable short-term clinical outcomes in arthroscopic osteophyte debridement for elbow osteoarthritis. Further studies with larger cohorts and extended follow-ups are necessary to optimize techniques and validate long-term efficacy.

Po6_2.08

Automatic Segmentation of Forearm Bones Using Deep Learning Approaches.

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Context : The segmentation of forearm bones (radius and ulna) in medical images is essential for applications such as fracture diagnosis, surgical planning, 3D printing, and monitoring musculoskeletal pathologies. **Objectives :** This project aims to develop an automatic method for segmenting forearm bone structures using various deep learning techniques, enabling precise analysis of CT images.

Methods : 79 CT scans were manually annotated, constituting the reference annotations. Among them, 64 volumes were used for training, and 15 for testing. Models were evaluated, including the pre-trained TotalSegmentator model and nnU-Net, which was trained on our dataset. The nnU-Net method demonstrated the highest performance. The obtained segmentations were compared to the manual segmentations using the Dice metric and the Average Symmetric Surface Distance (ASSD).

Results : Preliminary results show that the nnU-Net model achieved an average Dice score of 0.9721, with values ranging from 0.9606 to 0.9811. The mean ASSD was 0.3304, with values varying between 0.2188 and 0.7141. Figure 1 presents visualizations of the segmentation results, while Table 1 summarizes the obtained performances.

Conclusion : These results demonstrate that the nnU-Net deep learning method enables precise and automatic segmentation of forearm bones, thereby facilitating the creation of a model for diagnosis, decision support, and planning in emergency settings where the time between patient admission and surgical intervention is limited.

Po6_2.09

When Navigating TKA, Tibia 1st Workflow Improves Functional Results Relative to Femur 1st Workflow, at 2Y Follow-up

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Abstract

This comparative study evaluates functional outcomes in total knee arthroplasty (TKA) performed with a navigation system using femur-first measured resection (MR) or tibia-first gap balancing (GB) surgical workflows. A single surgeon at one center conducted all procedures using the same implant and navigation system. Data from 123 patients, including demographic information and Oxford Knee Scores (OKS) at preoperative, six weeks, one year, and two-year follow-ups, were analyzed. Results showed no significant differences in early postoperative outcomes between workflows. However, from one year onward, the GB group demonstrated superior functional results, with an OKS improvement of 5.8 points at two years, exceeding the MCID. The GB approach, which integrates joint laxity data into femoral planning, may offer better joint balance and antero-posterior stability over time.

Po6_2.10

3D assessment of the hip joint of a patient with diffuse idiopathic skeletal hyperostosis (DISH) based on CT image

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Abstract

While diffuse idiopathic hyperostosis (DISH) has a predilection for the thoracic spine and is not associated with limb joint disorders, few reports have investigated the disease in detail. In this study, we investigated the association between ossification of spinal ligaments and ossification around the hip joint in patients with DISH by CT. Among patients who had CT from the neck to the pelvis from 2009 to 2018, 113 had DISH localized to the thoracic spine (T-DISH) and 43 had DISH extending to the lumbar spine (T/L-DISH), 56 patients did not meet Resnick's criteria but had a certain amount of ossification between three vertebrae. (This was defined as Pre-DISH.) In these patients, the presence of periprosthetic ossification, narrowing of the joint space, lateral center edge angle (CE), anterior CE angle, and posterior acetabular sector angle (PASA) were statistically analyzed. Ossification increased significantly in the superior and posterior hip joints with progression from Pre-DISH, T-DISH, and T/L-DISH. The joint fissure was predominantly narrowed bilaterally in Pre-DISH and T/L-DISH, and T-DISH and T/L-DISH. With the development of DISH, especially when DISH extends to the thoracolumbar spine, there is increased ossification around the hip joint, with parameter changes and narrowing of the joint space